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EFFECTS OF CHOLINERGIC PERTURBATIONS ON NEUROMOTOR - COGNITIVE PERFORMANCE

Final Report

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Atropine dosing ranging from 0.5 to 4.0 mg demonstrated significant cognitive-neuromotor impairment effects in the 2.0 and 4.0 mg dose. The effects shown after the 4.0 mg dose was of a much greater magnitude and duration than that of the 2.0 mg dose. However, all doses of atropine induced significant tachycardia which declined much more rapidly than performance measures.

The pharmacokinetics of I.M. atropine can be best described by a two-compartment with very fast first order adsorption. For all doses, atropine plasma levels and heart rate changes closely overlapped throughout the time course. In contrast, the differential time course of changes in atropine levels and behavioral impairment indicates that pharmacokinetics is not the primary rate limiting mechanism for brain effects of atropine. Potential differences in time course effects on M_1 vs M_2 receptors raise the question of whether some of the protective effects of atropine may not be maintained as long as other effects

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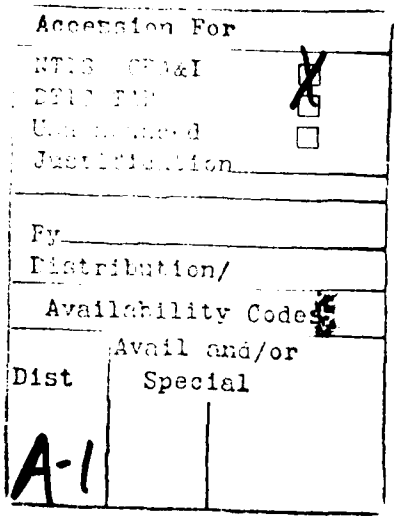
A variety of tasks of cognitive-neuromotor function were developed and used to test for atropine impairment. Test results involving complex psychomotor speed showed impairment over prolonged duration and demonstrated minimum relation to plasma time course of atropine. Coordination tasks such as subcritical tracking demonstrated a faster offset of action and had a more consistent relation of effect to plasma level. A variety of other tasks involving coordination, motor speed, and memory were tested and found not to be as robust in documenting impairment as the original task battery.

Foreword

For the protection of human subjects the investigators have adhered to policies of applicable Federal Law 45CFR46.



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Part I: Time Course of the Atropine Effect for CNS and Physiological Functions

Introduction

Atropine sulfate is currently distributed to military personnel as an antidote for anticholinesterone organophosphorus poisons (nerve agents) for the purpose of first-aid treatment (11). Due to the highly stressful and complicated nature of the tasks performed by soldiers and the likelihood that more than one injection will be self-administered in the event of a chemical attack, a comprehensive and detailed description of the magnitude and time course of the adverse effects of higher atropine doses is critical. This information is also needed to treat instances of premature injections or drug misuse in the absence of any organophosphorus nerve agent (11) as well as the abuse of atropine in recreational settings.

The complex nature of the pharmacologic effects of atropine is evident in the following results of previous studies: A) differential sensitivity to atropine for visual functions (2,9,12) and a wide variety of cognitive and psychomotor tasks (1,11), B) conflicting data on the dose-dependent alterations of physiological processes, including temperature, blood pressure and heart rate (2,5,9,14,20), and C) differential time course of the drug effect for certain physiological measures and other peripheral and central nervous system (CNS) capacities (2,5,9,12,14,20). The third finding was suggested by several studies that reported an earlier onset of effect and peak impairment and more rapid recovery from impairment for heart rate and blood pressure than for visual, cognitive and psychomotor measures, especially at doses of 2 mg or higher (2,5,9,11,12,14,20).

The inconsistent nature of the atropine effects poses an intriguing, and as yet unexplained, problem. Is impairment due to general sedative effects or to muscarinic block of selected areas of the peripheral and central nervous systems? Seppala and Visakorpi (20) argued that performance decrements induced by 1.7 mg could not be attributed to non-specific depressant effects since drowsiness was infrequently reported by their subjects. Thus, fatigue may not be the sole or primary mechanism underlying the CNS effect of atropine.

To elucidate further the central and peripheral effects of atropine, the present paper A) describes more precisely the time course for the onset of, and recovery from, impairment for several cognitive, neuromotor and physiological measures, B) compares the time courses of the behavioral and physiological drug effects and C) examines the relationship of drug effect to task difficulty and the nature of the task. The first aim is important since the majority of past studies evaluated the atropine effect at only one to three time intervals, ranging in length from 30 min to several hr (1,2,5,16,17,18,19,20). Moreover, except for a few reports (9,12,14), researchers generally continued observation of the drug effect for 4 hr or less. Thus, the present experiments were designed to monitor atropine induced impairment more frequently over a longer span of time. Finally, to evaluate the safety of administering higher doses of atropine, lower doses of 0.5, 1

and 2 mg were tested in Experiment I prior to increasing the upper dosage to 4 mg in Experiment II.

Materials and Methods

Experiment I

Subjects

Eight normal male volunteers, who were within ±10% of ideal height-weight ratios and had a mean age (±1 SD) of 24.4 (±2.1) yr, completed the study. All subjects were screened for serious physical and psychological problems and a history of drug abuse by physical and psychiatric evaluations, blood tests, urinalysis, glaucoma tests and electrocardiogram. A chemistry (with hepatic) profile and complete blood count were included among the blood tests and a urine screen for marijuana in the urinalysis. The Vocabulary, Block Design and Digit Symbol Substitution subtests of the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (23) and the Minnesota Multiphasic Personality Inventory were also administered to assess intellectual and emotional status. After completion of the study, the subjects were given a second physical examination. Informed written consent was obtained after the purpose and procedure of the study and the potential side effects of atropine were explained.

None of the subjects had any serious medical or psychological disorder or were taking anticholinergic or CNS-active drugs while in the study. All reported that they were nonsmokers and did not drink alcohol excessively. The mean (±1 SD) scaled scores for the Vocabulary, Block Design and Digit Symbol Substitution subtests were 12.9 (±1.6), 12.4 (±1.7) and 11.7 (±3.2), respectively.

Procedure

During three or four 2-hr sessions the subjects received training on the behavioral tasks until they showed no substantial improvement in performance. After the training phase, a sensitivity session was conducted to screen for extreme atropine reactivity which was not found in any of the subjects. An initial injection of 0.25 mg of atropine was followed by 0.25 and 1 mg at 40 min and 1 hr and 40 min, respectively. Performance and physiological measures were assessed at various intervals for approximately 7 hr following the initial drug administration. Four test sessions were then scheduled at 2-week intervals for subjects who had no unusual adverse reactions.

Subjects were instructed to sleep their normal number of hours the night before each test session and to consume no alcohol or drugs during the previous 24 hr. After reporting to the laboratory at 7:30 A.M., subjects were given a light breakfast, and predrug performance and physiological measurements were collected. Using a double-blind procedure, placebo or a single atropine dose of 0.5, 1 or 2 mg was injected intramuscularly (I.M.) 1 hr after breakfast. Following drug administration, tasks and physiological measurements were repeated at 10, 15, 35, 55, 75, 95, 115, 135, 165, 210, 230, 330, 350, 380, 400 and 430 min. Order of drug administration was determined

by a random Latin square design. All subjects ate a standard lunch of a meat (usually turkey) sandwich and noncaffeinated soda.

Individual syringes of atropine sulfate or placebo were prepared by the Duke University Medical Center Pharmacy. Placebo consisted of 2 ml of bacteriostatic water. The atropine dose in each syringe was diluted with sufficient quantities of bacteriostatic water to produce a volume of 2 ml. During the sensitivity session, single syringes containing 0.25 or 1 mg were used. During the test sessions two syringes were used for each dose and one syringe was injected into the ventral aspect of each upper thigh. The contents of the syringes were as follows: two syringes of placebo for the placebo condition; one syringe of 0.5 mg of atropine and one syringe of placebo for the 0.5 mg dose; one syringe of 1 mg of atropine and one syringe of placebo for the 1 mg dose; one syringe of 2 mg of atropine and one syringe of placebo for the 2 mg dose.

Performance Tasks

The experimental chamber, apparatus and all of the following tasks, except for continuous performance and divided attention, have been described in prior reports (6,7,8). Testing was conducted in a quiet room which was darkened for all tasks, except for the sway task with eyes open or closed.

Continuous subcritical tracking (SCT). A 3-cm wide vertical bar was projected down the middle of a 98 x 128-cm video screen. A 24-cm segment from the center of the bar moved laterally across the screen and the subjects maintained the moving bar in alignment with the stationary portions of the bar for 3 min by turning a car steering wheel. During the first 20 sec the degree of difficulty gradually increased to the easy level of lambda 2 and remained at lambda 2 for 60 sec (easy SCT). Lambda then rose to 3 over the next 20 sec and stayed at 3 for 80 sec (hard SCT). The initial, middle and final 20 sec of data were ignored. Thus, the dependent measure was based on data collected for 1 min at each lambda and expressed in root mean squared (RMS) units, that is, the square root of the mean of the squared deviations from the center position.

Standing steadiness. The sway table consisted of 2 steel platforms, 45.5 x 45.5 x 1 cm, separated by four steel bars which were positioned at right angles to each other. A pair of strain gauges attached to each bar transduced lateral and anterior-posterior movements into varying voltages which were digitized and analyzed using Fast Fourier transforms. On two ataxia tasks the subjects stood on the sway table for 30 sec and either looked at a fixed point in front of them (eyes open) or closed both eyes (eyes closed). The overall measure of ataxia was the square root of the sum of the power scores for frequencies below 2.5 Hz.

For the standing steadiness with visual feedback task, a circle was drawn on the screen and a "+" symbol in the center of the circle represented the center of balance for individual subjects. The standing positions of the subjects were sampled 30 times per sec and indicated on the display for the previous 1 sec by a series of 30 dots. The subjects attempted to minimize swaying for 60 sec by keeping the dots as close as possible to the "+".

Anterior-posterior and lateral motions were measured and the mean distance from center was computed.

Digit symbol substitution (DSS). In a computerized modification of the Digit Symbol Substitution subtest of the WAIS-R, the code table of nine randomly paired symbols and numerals was displayed on the screen. As each symbol appeared below the table, the subjects pressed on a 9-digit keypad the number paired with that symbol. There were 12 presentations per symbol. Subjects were trained to respond quickly and accurately. The response measure, total power, represented the ratio between accuracy and speed and was calculated by dividing total number of correct responses (NC) by the average reaction time (RT) for correct responses.

Digit symbol substitution task with short-term memory (DSSM). The procedures and analyses of the DSS and DSSM tasks were identical, except for the following changes. The DSS part of DSSM was divided into four quartiles with 3 presentations of each symbol per quartile. During a recall test at the end of each quartile the code table was erased, the symbols were displayed individually and the subjects pressed on the keypad the number paired with each symbol in the current code table. The dependent measures included total power for the DSS portion and total NC and mean RT for the four recall tests.

Keypad reaction time (KRT). Numbers from 1 to 9 were randomly selected and displayed individually in the center of the screen for 108 trials. The subjects pressed the same number on the keypad as quickly and accurately as possible. The response measure was the same as for the DSS task.

Continuous performance. Individual digits from 1 to 9 were randomly selected. Each of the first 54 numerals was shown for 2 sec and each of the second 54 numbers for 1.33 sec. The duration of the task was 3 min. The subjects pressed any key on the keypad as quickly as possible when an odd followed an even number or an even followed an odd number. Half of the trials involved even-odd or odd-even number sequences, while the other half were even-even or odd-odd sequences. The dependent variable was the same as for the DSS task, except that NC was the difference between the number of right and wrong responses.

Divided attention. The divided attention task consisted of the simultaneous presentation of the SCT and continuous performance tasks. Since the continuous performance portion was the secondary or distractor test, only this task was modified. The 108 digits were presented at a constant rate of one every 1.67 sec and the numerals were displayed at the top of the screen so that they would not interfere with the SCT bar. The duration of the task was 3 min. The response measures were the same as for the SCT and continuous performance tasks.

Physiological Measurements

Heart rate was monitored continuously using a 7830A Hewlett Packard heart monitor for identification of the onset of bradycardia or tachycardia. Pulse while sitting and standing was recorded as beats per min by counting the auditory beats from the monitor for 30 sec and multiplying by 2. Oral

temperature was assessed using a test probe, the Sensortek Thermometer and Model TH-8 Temperature Monitor and Bailey Probe Selector.

Experiment II

Subjects

The characteristics of the 16 young males in the second study were similar to the subjects in Experiment I. They had a mean age (±1 SD) of 24.1 (±1.2) yr and mean scaled scores (±1 SD) of 15.3 (±2.4), 13.9 (±3.4) and 12.0 (±2.9) for the Vocabulary, Block Design and Digit Symbol Substitution subtests, respectively. As in Experiment I, screening tests were conducted and written informed consent was obtained. A treadmill test was added to the screening protocol.

Procedure

The experimental protocol, performance and physiological measures, and analyses were identical for both experiments, except for the following changes. First, the 0.5 mg dose was replaced by 4 mg due to the lack of a substantial drug effect for the lower doses in Experiment I. Two syringes, each of which contained 2 mg of atropine, were used for the 4 mg dose. For the sensitivity session, the dosing schedule was altered to an initial dose of 0.5 mg at 0 min, followed by 1 mg at 40 min and 1.5 mg at 1 hr and 40 min after the first injection. Second, to minimize human error in measuring heart rate, the Digital 11/23 computer was programmed to calculate heart rate as beats per min from the analog output of the heart monitor and was within ±1 beat per min of the independent measurement of heart rate by palpitation.

Data Analysis

Results of a multivariate analysis of variance test indicated that the subjects of the two studies did not differ significantly on the prestudy characteristics: age, weight, height, and scores on the Block Design, Digit Symbol Substitution and Vocabulary subtests. Predrug scores were not significantly correlated with study or drug treatment condition. Finally, since there were no significant differences between the two experiments on the postdrug scores of the dependent measures for 1 and 2 mg, the data from both studies were combined for the following analyses.

As discussed in the introduction, the present studies were designed to test for the significance of the pharmacological effects of several doses of atropine over time. T-tests were conducted to determine the significance of the *a priori* or planned comparisons, that is, the difference between the mean scores of each dose condition and that of placebo at each testing time point. The means were adjusted for the predrug score. The dose conditions were 0.5, 1.0, 2.0 and 4.0 mg of atropine. The dependent variables were the scores of the behavioral and physiological measures. To linearize the relationship between independent and dependent variables, logarithmic transformations of the SCT scores and square root transformation of the measures for the sway with eyes open or closed tasks were conducted and used in the t-tests. To protect the experimentwise α level, the modified Bonferroni method (13) was

used to correct the p values of the multiple t-tests by dividing the overall α by the total number of t-tests performed for each dependent measure.

Results

As shown in Figs. 1 to 4 and Table I, a dose-dependent effect was evident for the various tasks. There was a significant effect for 2 and 4 mg on the SCT, standing steadiness with eyes open, DSSM, continuous performance and KRT tasks and for 4 mg only on the DSS and divided attention tasks and RT of the DSSM recall tests. The impairment magnitude produced by 4 mg on the behavioral tasks was substantially greater than the level of effect associated with the lower doses.

The SCT, DSS and standing steadiness with eyes open tasks were presented more frequently than the other tasks during the initial 2 hr of testing to obtain a more detailed picture of the changes in the nature of the drug effect during this early period. The SCT task was assessed at all sixteen postdrug performance testing time intervals, the DSS and sway with eyes open tasks at all but the 10-min time point and the remaining tasks at 135, 165, 230, 350 and 400 min. The onset of the drug effect was characterized by a slight delay for the SCT task and by a relatively late initial mean impairment peak at 75 min for the SCT and DSS tasks after 4 mg and at 95 min for the SCT task after 2 mg (Figs. 1 and 2). The mean maximum impairment peak after 4 mg for the DSS task was observed even later at 115 min.

On the SCT and DSS tasks, the initial impairment peak for the 4 mg dose was followed by a plateau phase which had a sustained level of impairment. The length of the plateau phase varied from about 40 min for the easy SCT task to 2.5 hr for the DSS task. The plateau phase was also present in the impairment profiles induced by 4 mg for the DSSM, KRT and continuous performance presented alone tasks (Fig. 3 and Table I).

After the plateau phase the tasks demonstrated differential rates of recovery from impairment. A relatively faster rate was observed for the SCT task (Fig. 1) than for cognitive tasks which involved psychomotor speed, including the DSS, DSSM, KRT and continuous performance tasks (Fig. 2 and 3). While the SCT and cognitive tasks were still significantly impaired by 4 mg at the time of the final assessment at a little over 7 hr post drug administration, SCT performance alone attained recovery levels that were close to predrug levels at the end of the test session.

In contrast, a significant increase in ataxia was produced by the 4 mg dose at only 135 and 210 min for the eyes open condition ($p<0.003$) and at 165 and 230 min for the eyes closed condition ($p<0.01$). The subjects also became ataxic briefly following the 2 mg dose at 135 min on standing steadiness with eyes closed. No significant drug effect was found for the sway task with visual feedback.

The total number of digit-symbol pairs recalled correctly on the 4 DSSM recall tests was significantly ($p<0.01$) decreased by the 4 mg dose at 135 and 165 min. On the other hand, the subjects continued to require a significantly

Table I

Mean Power Scores of Cognitive Tasks After Atropine Injection[a]

Tasks	Postdrug Assessment Times (min) 135	165	230	350	400
DSSM					
Placebo	11.1	8.0	7.0	6.7	4.5
2 mg	17.9	15.6*	13.8	11.8	12.5
4 mg	23.6**	23.7***	24.3***	19.1**	20.3***
KRT					
Placebo	7.4	3.6	1.2	1.2	-0.9
2 mg	23.6*	13.9	14.7*	6.6	1.4
4 mg	33.2***	32.3***	35.2***	29.5***	23.7*
Continuous performance - alone					
Placebo	0.3	1.9	0.7	1.0	0.9
2 mg	12.3**	13.4**	11.0**	5.7	7.5
4 mg	23.0***	22.8***	22.1***	19.3***	17.6***
Divided attention - continuous performance					
Placebo	1.0	0.6	2.3	1.4	-1.0
4 mg	12.3**	15.8***	14.9***	10.3**	7.0**

[a]The power score is explained in the text. Mean delta scores or unit changes from the predrug score are shown. The DSSM scores are the power scores for the DSS part of the DSSM task. Only results for placebo and atropine doses that induced significant impairment are presented. *, ** and *** represent $p<0.01$, $p<0.002$ and $p<0.0002$ significance levels, respectively, for Bonferroni *t*-tests used to compare dose vs. placebo treatments.

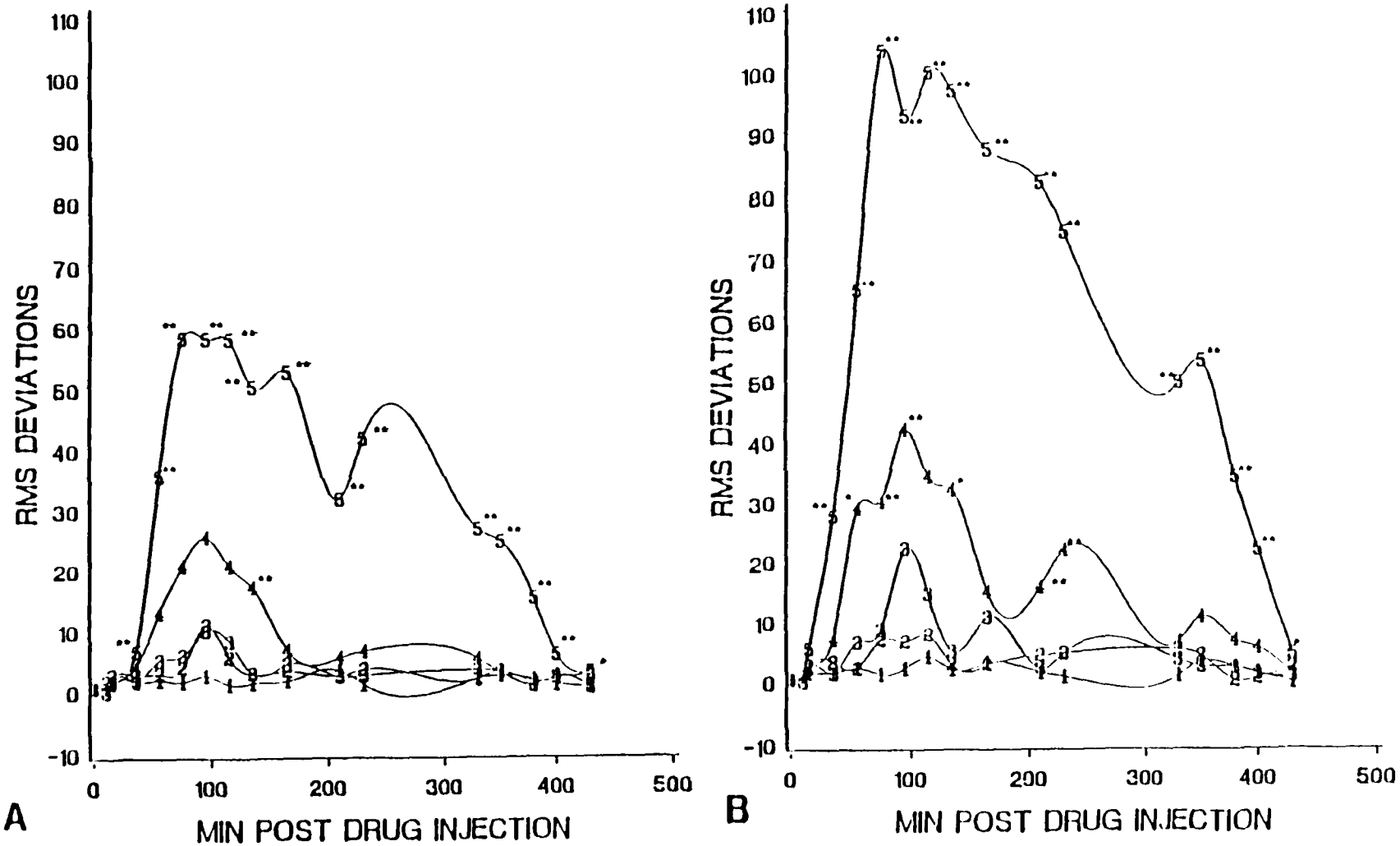

Figure 1. Performance on the SCT task after I.M. injection of placebo (1) and 0.5 (2), 1 (3), 2 (4) and 4 (5) mg of atropine. Impairment of performance on the easy (A) and hard (B) difficulty levels are shown. Performance is represented as the mean number of unit changes from the predrug score (mean delta scores). A smooth spline fitting procedure was used to connect sequential testing time points. * and ** represent $p<0.003$ and $p<0.0006$ significance levels, respectively, for differences between placebo and each atropine dose.

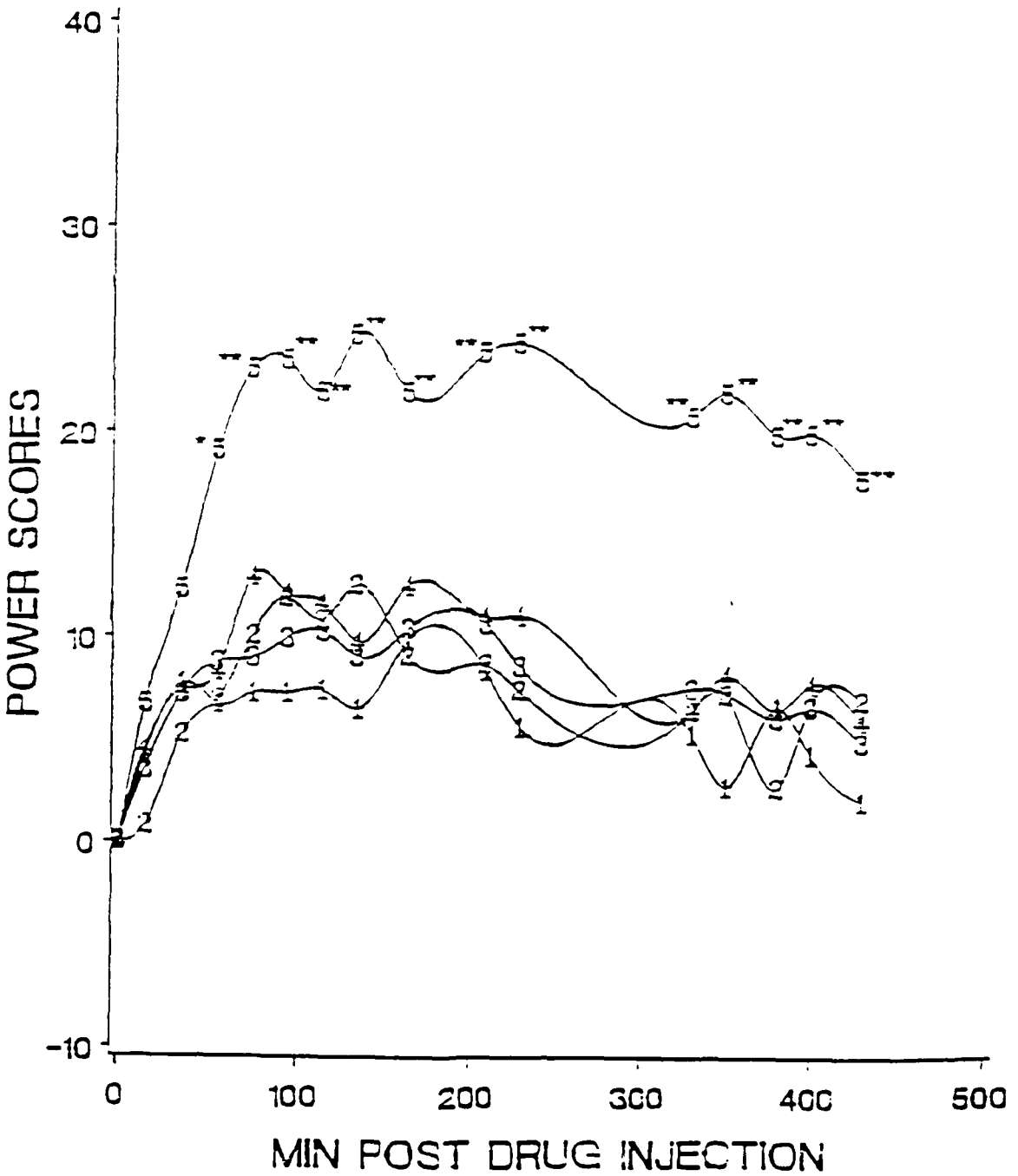

Figure 2. Performance on the DSS task following I.M. injection of placebo (1) and 0.5 (2), 1 (3), 2 (4) and 4 (5) mg of atropine. Performance is represented as mean delta scores. The delta scores, unit of measurement, curves and significance levels are described in Fig. 1 and the text.

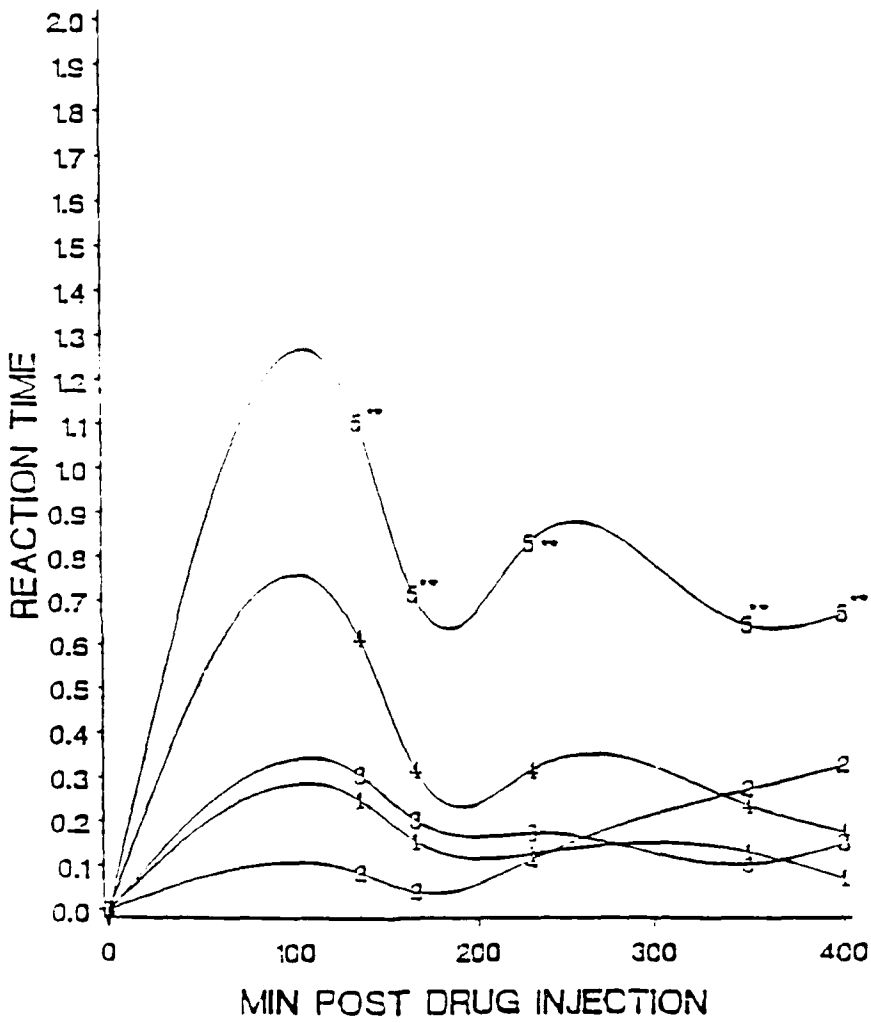

Figure 3. Performance on the four recall tests of the DSSM task following I.M. injection of placebo (1) and 0.5 (2), 1 (3), 2 (4) and 4 (5) mg of atropine. Performance is represented as delta scores of the average reaction time for responses on the memory tests. A description of the delta scores, unit of measurement, and curves is presented in Fig. 1 and the text. *, **, *** represent $\underline{P}<0.01$, $\underline{P}<0.002$, and $\underline{P}<0.0002$ levels of significance, respectively, for the Bonferroni t-tests used to compare dose vs. placebo treatments.

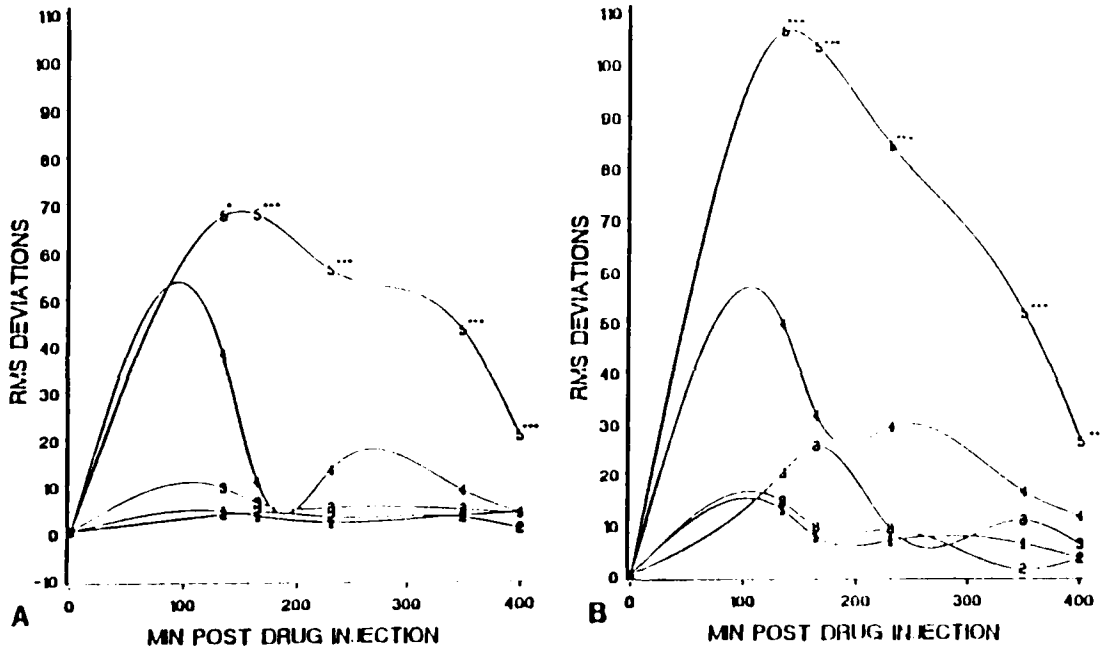

Figure 4. Performance on the easy (A) and hard (B) SCT portions of the divided attention task after I.M. injection of placebo (1) and 0.5 (2), 1 (3), 2 (4) and 4 (5) mg of atropine. Performance is represented as mean delta scores. The delta scores, unit of measurement, and curves are explained in Fig. 1 and the text. The significance levels are the same as in Fig. 3.

($p<0.01$) longer response time to recall the digit-symbol pairs at all five postdrug assessment times during the 4 mg test session (Fig. 3).

The hypothesis of greater drug sensitivity as task difficulty increases is supported by the finding of higher impairment magnitude after 2 and 4 mg for the hard SCT than for the easy SCT task (Fig. 1). Moreover, impairment was noted for a longer time by the 2 mg dose during the hard SCT task. Similarly, 4 mg produced greater impairment of the hard than of the easy SCT portion of the divided attention task (Fig. 4). The rate of recovery from impairment appeared to be similar for the easy and hard SCT tasks, presented alone or as part of the divided attention task.

As for the behavioral tasks, the effect of atropine on sitting heart rate demonstrated a dose-dependent relationship (Fig. 5). Unlike the CNS tasks, however, as the dose was increased, there was a proportionate increase in the magnitude of the effect on heart rate. All four doses induced a significant increase in heart rate, but in general, the higher doses produced a more pronounced effect and a slower recovery rate. Compared to the SCT and DSS tasks, the atropine effect on heart rate had a faster onset and reached its maximum effect within 1 hr after I.M. injection. Recovery from the drug effect also occurred more rapidly for heart rate. A similar time course of drug effect was found for standing heart rate. Oral temperature was not significantly altered by any of the doses.

Discussion

In general, the behavioral and physiological measures demonstrated differential degrees of drug sensitivity that were dose-related. While all four atropine doses significantly increased heart rate (Fig. 5), just the highest dose produced substantial and prolonged impairment of the behavioral tests (Figs. 1 to 4, and Table I). More importantly, whereas each higher dose produced a proportionate increase in the magnitude of the heart rate effect, 4 mg resulted in a dramatically greater increment in impairment than any other dose on most of the CNT tasks. Although Haegerstrom-Portnoy et al. (9) observed no differences in the magnitude of the pulse rate changes produced by 2 and 4 mg, others agreed with the present studies and reported dose-dependency as a common characteristic of both physiological and central atropine effects (5,11,18,20). The finding of much greater behavioral impairment at 4 mg is especially significant since more than one 2 mg dose is likely to be self-administered by soldiers under combat conditions.

Differences between the physiological and central drug effects are also evident in the shapes of the impairment profiles over time. Compared to the heart rate measure, the onset of the impairment of the SCT and DSS tasks was slower, resulting in a later maximum impairment peak for the behavioral tasks. In addition, the effect of atropine on the behavioral tasks continued for a much longer period of time than for the physiological variable. While heart rate was accelerated significantly for approximately 5 1/2 hr by 4 mg, performance on many of the cognitive and neuromotor tasks was still significantly impaired at the final testing time point which was over 7 hr following drug administration. Perusal of the time course profiles further reveals that performance on tasks involving psychomotor speed and memory

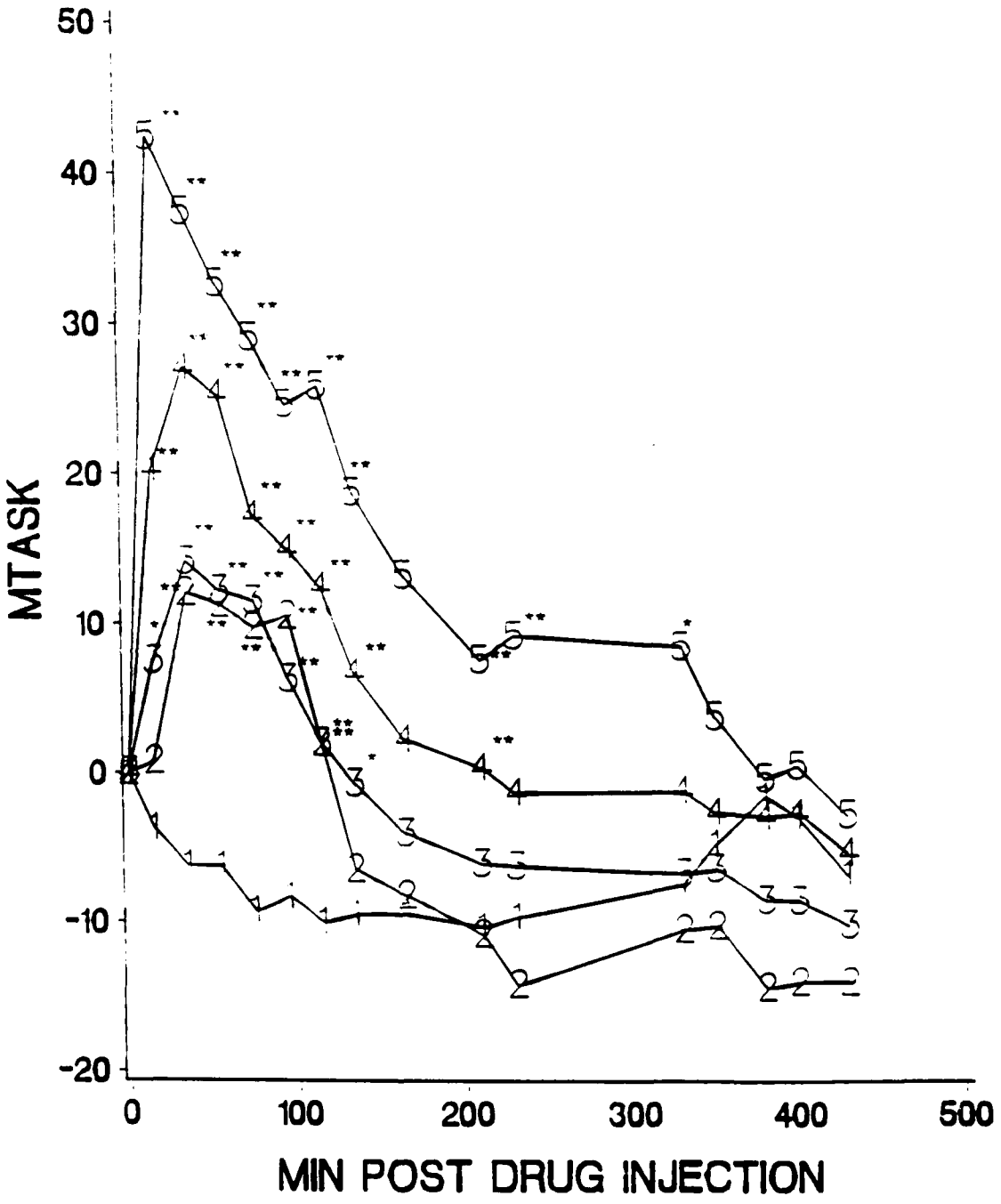

Figure 5. Sitting heart rate measurements expressed as beats per min (bpm) after I.M. injection of placebo (1) and 0.5 (2), 1 (3), 2 (4) and 4 (5) mg of atropine. Heart rate is represented as mean delta scores. The delta scores, unit of measurement, curves and significance levels are specified in Fig. 1 and the text.

encoding improved at a considerably slower rate after maximum impairment than on coordination based tasks (Figs. 1 to 4) and that the rate of recovery from the atropine induced tachycardia was much faster than for either type of task (Fig. 5).

In view of the dramatically differential impairment time courses for the central and peripheral types of effects, an important question is how the underlying mechanisms mediate the different effect time courses. A major contributor to these differences may be receptor heterogeneity. High densities of high affinity pirenzepine, [^{3}H]Pz binding sites (so called Muscarinic M^1 sites) are found in the cerebral cortex (21,22), corpus striatum and hippocampus, while low affinity [^{3}H]Pz binding sites (M^2 sites) predominate in the cerebellum, medulla-PONS and peripheral tissues, such as the heart and ileum. Pirenzepine has as much as 40 times the antagonist potency in high affinity sites versus the cardiac atrial sites (4,10,24). Atropine, which was used in the present study has relatively equal potency for M^1, M^2 sites. Characteristically, the M^1 site mediates the inhibition of the slow M-current, generated by a voltage-sensitive potassium channel (3). This M-current excitatory action is very slow in onset and has a prolonged duration (15), contrasted with the faster peripheral action of acetylcholine. Thus, both anatomical and receptor pharmacodynamic evidence are consistent with the hypothesis that receptor heterogeneity contributes to the differential time course of atropine effects.

A close examination of the results for the SCT task also provides evidence of an interaction between drug effect and task difficulty. Results provide evidence that work at the hard difficulty level was significantly impaired by 2 and 4 mg, but at the easy level was sensitive for a long time only to the higher dosage (Figs. 1A and 1B). Moreover, the mean maximum impairment value was considerably greater for the hard SCT version. On the other hand, the increase in task demands did not seem to affect the rate of recovery from impairment. Parallel curves were observed during the effect offset phases for both versions of the SCT task and for the SCT task, presented alone or as part of the divided attention task (Figs. 1 and 4). The greater difficulty of the divided attention SCT task is indicated by the finding that during the placebo condition the hard tracking scores from the divided attention task were almost or more than twice as great as the scores of the SCT task performed alone.

The time course for the therapeutic effect cannot be determined from the behavioral and physiological curves described in the present report. Nevertheless, these curves should be considered in deliberations about CNS vs. physiological coverage for cholinesterase poisoning. The present findings raise the concern that repeated dosing may be required for physiological or peripheral coverage, yet at the same time would result in accumulative central impairment. Whereas there is greater individual variability at lower doses, a greater number of persons will consistently show substantial impairment at higher atropine concentrations and the risk of performance failure will be increased. Finally, the discrepancies between the time course profiles for the SCT, sway and cognitive tasks during the drug offset phase suggest that complex tasks involving psychomotor speed and/or memory will result in more prolonged impairment than tasks requiring primarily motor coordination skills.

If receptor heterogeneity is the basis for the differential time course of effects, then the development of specific muscarinic antagonists would facilitate treatment and prophylaxis of anticholinesterase poisoning.

Summary

Atropine is widely used as an antidote for anticholinesterase poisoning, pre-operative medication and treatment for conditions, such as peptic ulcers and Parkinsonism. Two studies were conducted to examine the effect of atropine on cognitive, neuromotor and physiological measures. Single doses of placebo and 0.5, 1, 2 and 4 mg of atropine were administered intramuscularly to healthy young male volunteers according to a random Latin square design. Performance tasks included wheel tracking, standing steadiness, digit symbol substitution, reaction time, divided attention and memory tasks. While all four doses significantly increased heart rate, only 2 and 4 mg, induced significant impairment of performance on the cognitive and neuromotor tasks. Duration of the drug effect was also more prolonged for the behavioral tasks than for heart rate. Task difficulty and psychomotor speed were associated with increased sensitivity to atropine. Potential pharmacodynamic mechanisms and clinical implications of the findings are discussed.

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Part II: Comparison of CNS and Peripheral Pharmacodynamics to Atropine Pharmacokinetics

Introduction

Despite the clinical use of atropine for many years (Weiner, 1985), relatively little data is available on the relationship between atropine pharmacokinetics and pharmacologic activity. A few studies have examined the association between drug levels and effect for physiological variables (Hinderling et al., 1985a), but there has been no systematic attempt to examine the same relationship for effects on cognitive and psychomotor performance. The importance of understanding the connection between pharmacokinetics and behavioral effects is highlighted by the observation of differential time courses for certain physiological and central nervous system (CNS) effects of atropine.

Previous studies have found that the onset of the atropine effect and peak impairment occurred earlier for heart rate than for measures of pupil dilation and accommodation and performance on cognitive, tracking and number facility tasks at doses of 2 mg or higher (Haegerstrom-Portnoy et al., 1987; Headley, 1982; Ketchum et al., 1973). Atropine induced increases in heart rate and blood pressure also subsided more quickly than impairment of visual, cognitive and neuromotor abilities (Ketchum et al., 1973). Furthermore, CNS syndromes, including amnestic delirium, which are produced by atropine intoxication often have a more prolonged duration than is indicated by the short elimination half-life of atropine. The differential properties of the atropine effects on the cardiovascular and CNS measures raise important questions about the interaction and the relative primacy of pharmacokinetic and pharmacodynamic mechanisms.

In preliminary studies, we found that time course changes in plasma levels following an acute dose of atropine fails to define the onset and offset of the CNS effects (Gupta and Ellinwood, 1987; Nikaido et al., submitted). Both pharmacokinetic and pharmacodynamic mechanisms can be proposed to explain this discrepancy and include: 1) equilibration rate between blood and CNS or distribution kinetics, 2) drug receptor dissociation rate differences based on receptor affinity, 3) receptor kinetic and transduction differences in heterogenous receptor populations. Thus, muscarinic receptor heterogeneity, the subject of many recent publications, may certainly be a primary determinant of the lack of correspondence between the time course of the CNS effect and that of both the peripheral effect and changes in plasma atropine concentration.

The receptor heterogeneity hypothesis is supported by data from studies of both the anatomical distribution of receptors and receptor pharmacodynamics. Whereas high affinity pirenzepine ([^{3}H]Pz) binding sites which are also called muscarinic M_1 sites, are present in large concentrations in the cerebral cortex (Watson et al., 1982; Watson et al., 1983), corpus striatum and hippocampus, there is a predominance of low affinity [^{3}H]Pz binding (M_2) sites in cerebellum, medulla-PONS and peripheral tissues, including the heart and ileum. Pirenzepine in high affinity sites has up to 40 times the antagonist potency than in cardiac atrial sites (Brown et al.,

1980; Hammer and Giachetti, 1982; Wess et al., 1984), while atropir demonstrates relatively equal potency for the M_1, M_2 sites. An important characteristic of the M_1 sites is the inhibition of the slow M-current which is generated by a voltage-sensitive potassium channel (Brown and Adams, 1980). In contrast to the faster peripheral activity of acetylcholine, the excitatory action of the M-current has a very slow onset and a longer duration (Krnjevic, 1974).

The present experiments were conducted to investigate further the above questions. The results from these studies on the magnitude and time course of the atropine effect for a variety of behavioral and physiological variables have been described in another paper (Nikaido et al., submitted). As in prior studies (Haegerstrom-Portnoy et al., 1987; Headley, 1982; Ketchum et al., 1973), differences were observed in the time course profiles of the CNS and cardiovascular impairment. The primary aim of the current report is the comparison of the concentration-effect relationship for the two types of measures in order to assess the relative contribution of pharmacokinetic and pharmacodynamic parameters.

The following presentation focuses on three of the measures which were selected because they were highly sensitive to the atropine effect and allowed us not only to compare the central vs. peripheral effects, but also to consider the results in terms of M_1 vs. M_2 receptor sites. Moreover, due to the time limitations set by the length of the tasks, the three measures were assessed more often than others during the initial 2 hrs of testing (described in Nikaido et al., submitted) so that a sufficient number of data points could be collected for an adequately detailed depiction of the association between drug effect and pharmacokinetics during the early impairment phase.

As discussed above, prior research suggests that the peripheral M_2 type of receptor may be a key mediator of the atropine effect on heart rate, the physiological variable. The CNS functions were the subcritical tracking (SCT) and digit symbol substitution (DSS) tasks. The primarily neuromotor SCT task is hypothesized to be based on both extrapyramidal M_1 and cerebellar M_2 receptor types, while DSS, a cognitive task with memory and psychomotor components, may involve hippocampal-cortical-extrapyramidal M_1 receptors. The subjects, procedure, test room, task apparatus and the dependent measures for the following experiments have been described in greater detail by Nikaido et al. (submitted).

Materials and Methods

Experiment I

Subjects

Eight young men with a mean age (±1 SD) of 24.4 (±2.1) yr finished the study. The aims, procedure and potential risks of the experiment were described to the subjects and informed written consent was obtained. All of the subjects were healthy with no serious physical or mental illness. They were non-smokers, drank in moderation and did not use anticholinergic or CNS-active drugs during their participation in the study. Their mean scaled

scores (±1 SD) for the Vocabulary, Block Design and Digit Symbol Substitution subtests of the Wechsler Adult Intelligence-Revised (WAIS-R) (Wechsler, 1981) were 12.9 (±1.6), 12.4 (±1.7) and 11.7 (±3.2), respectively.

Procedure

After subjects were trained on the behavioral tasks, a sensitivity session was conducted to eliminate subjects who were highly sensitive to atropine. Doses of 0.25 mg, 0.25 mg and 1.0 mg of atropine were injected intramuscularly (IM) at 0, 40 and 100 min, respectively, and drug concentration and effect on task and physiological measures were monitored for 7 hours. No individuals demonstrated any unusual adverse reactions, in Experiments I and II.

A double-blind procedure was used to inject IM an acute dose of 0.5, 1.0 or 2.0 mg of atropine or placebo containing 2 ml of bacteriostatic water during each of the four test drug sessions. There was a 2-week washout period between sessions. The sequence of the drug treatments was assigned to the subjects according to a random Latin square design. Subjects arrived at the test room at 7:30 A.M., ate a small breakfast and had an obturated intravenous teflon catheter inserted in the forearm for drawing blood samples. Task and physiological assessments were performed and a blood sample was taken prior to drug administration. Tasks and heart rate were also assessed at 10, 15, 35, 55, 75, 95, 115, 135, 165, 210, 230, 330, 350, 380, 400 and 430 min and blood samples were drawn at 10, 20, 40, 60, 80, 100, 120, 160, 190, 255, 375 and 425 min after drug administration. Lunch was a meat sandwich and noncaffeinated soda and was eaten at 260 min postdrug injection.

Performance and Physiological Measures

Continuous subcritical tracking (SCT). A vertical bar extended from top to bottom in the middle of the 98 x 128 cm screen. A small section from the center of the bar moved horizontally across the screen and the subjects turned a steering wheel to keep the moving bar in the center of the screen for 3 min. During the first and second halves of the task, degree of difficulty was easy and hard, respectively. The deviations of the bar from the center were summarized as the square root of the mean squared error or root mean squared (RMS) deviation.

Digit symbol substitution (DSS). This task is a computerized modification of the Digit Symbol Substitution subtest from the WAIS-R. Nine randomly paired numerals (1 to 9) and abstract symbols comprised a code table that remained on the screen during the task. Each symbol was presented individually below the table 12 times and the subject pressed on a 9-digit keypad the number associated above that symbol on the table. The dependent variable was a total power score, which was calculated by dividing total number of correct responses (NC) by the average reaction time (RT) for right responses, and thus represented the relationship between speed and accuracy.

Heart Rate. The physiological measure, sitting heart rate, was recorded by counting the beat-to-beat audio output from a 7830A Hewlett Packard heart

monitor for 30 sec and multiplying the result by 2 to obtain the number of beats per minute.

Atropine Assay

Plasma atropine concentrations were determined by the radioimmunoassay method as described by Wurzburger et al. (1977) with modification. The lower limit of sensitivity was 0.25 ng/mL and the interassay coefficient of variation was about 6%.

Protein Binding Studies

Protein binding (both in vitro and in vivo) of atropine was determined in plasma, serum, human albumin (40 g/L), human alpha-1 acid glycoprotein (AAG, 0.7 g/L) and AAG spiked plasma and serum. Protein samples (1 ml each) were spiked with known amounts of atropine to yield concentrations between 0 and 20 ng/ml. Protein binding was determined by both ultrafiltration and equilibrium dialysis methods using both hot and cold atropine. Atropine concentrations were determined using the method above.

Growth Hormone Assay

Growth hormone (GH) concentrations in the plasma were determined with a human growth hormone assay kit (Sucrosep, Boots - Celltech Diagnostics Ltd., UK), using their standard radioimmunoassay method. The lower limit of sensitivity was 25 pg/mL and interassay coefficient of variation was 9.8%.

Experiment II

Subjects

The subjects were 16 young males who satisfied the same requirements that were used in Experiment I. Their mean age (±1 SD) was 24.1 (±1.2) yr and mean scaled scores (±1 SD) for the Vocabulary, Block Design and Digit Symbol Substitution subtests were 15.3 (±2.4), 13.9 (±3.4) and 12.0 (±2.9), respectively.

Procedure

The experimental protocol and performance, physiological and atropine measures were the same as for Experiment I. The major modification in the test sessions of the second study was the elimination of the 0.5 mg dose and the addition of a 4.0 mg dose since adverse reactions were infrequently observed in Experiment I. A computerized method of recording heart rate was introduced in Experiment II.

Data Analysis

As described by Nikaido et al. (submitted), there were no significant differences between the two studies for age, WAIS-R subtest scores, height, weight and scores on the tasks and heart rate for the 1 and 2 mg doses. Thus, the data of the two experiments were combined for the following analyses.

Plasma atropine concentration vs. time data for individual subjects were analyzed using both compartmental and non-compartmental methods. For the compartmental analysis, the initial estimates of the parameters and compartmental configuration were determined using ESTRIP (Brown and Manno, 1978). Parameter values were further refined using the SAS NLIN procedure (SAS User's Guide, 1982). The following pharmacokinetic parameters were determined for each subject: absorption (k_a), distribution (α) and elimination (β) half-life ($t_{\frac{1}{2}}$), total plasma clearance (CLp/F), mean residence time (MRT), total volume of distribution at steady state (Vdss/F), lag time, dose normalized maximum concentration (C_{max}), and unbound volume of distribution at steady state (Vdss/F, free). Analysis of variance (ANOVA) was performed to determine if there were significant differences between dose levels for appropriate parameters. Duncan's Multiple Range Test was used to determine the significance between pairs of means when ANOVA indicated that a significant difference existed.

To examine the relationship over time of atropine plasma levels to CNS and heart rate measures, hysteresis curves were constructed. For each task and heart rate, mean change in score from the predrug level was plotted as a function of the mean atropine concentration for the same testing time point. Drug concentration was estimated by using the pharmacokinetic parameters computed for individual subjects. Regression analyses were performed to calculate the amount of variability in the dependent measures that could be related to drug concentration. The regression analyses were conducted for each dose for the entire time course, from drug injection to the time of maximum impairment (drug effect onset phase) and from the maximum effect time point to the end of the test session (effect offset phase). Since the sample size for the 0.5 mg dose (N=8) was much smaller than for the higher doses (N=16 or N=24), the results for the lowest dose were more likely to be more variable than for the other doses and were therefore not included in the hysteresis or regression analyses.

Results

Atropine Pharmacokinetics

Average plasma atropine concentration as a function of time following IM administration of 0.5, 1.0, 2.0 and 4.0 mg of atropine as atropine sulfate monohydrate is shown in Fig. 6. The application of the F-test in the ESTRIP program indicated that individual profiles of the plasma atropine concentration vs. time relationships were best characterized by a two-compartment model with first order absorption with a lag time. Mean values for the kinetic parameters are summarized in Table II. As shown in Fig. 7A, the mean value of the area under the plasma vs. time curve (AUC) rose disproportionally in comparison to the increase in dose. There was also a disproportionate increase with dose in mean values of CLp/F and Vdss/F (Table II). The mean half-life ($t_{\frac{1}{2}}\beta$) and MRT (Fig. 7B) decreased accordingly upon increasing the dose. However, there was no change in unbound Vdss/F, regardless of dose level.

Atropine was found to be poorly bound (about 44%) to plasma protein, mainly to alpha-1 acid glycoprotein (AAG). Atropine binding to AAG was

Table II. Average Pharmacokinetic Parameters of Atropine Following Intramuscular Dosing

Parameters	Dose (mg)			
	0.5	1.0	2.0	4.0
$t_{\frac{1}{2}}ka$ (hr)[a]	0.08	0.06	0.07	0.06
Lag time[a]	0.11	0.10	0.11	0.12
MRT (hr)	4.31	4.08	3.40	2.61
Dose Normalized c_{max} (ng/ml)	2.40	2.31	1.80	1.69
$t_{\frac{1}{2}}\alpha$ (hr)	0.35	0.30	0.52	0.44
$t_{\frac{1}{2}}\beta$ (hr)	3.30	2.96	2.34	1.62
CLp/F (L/h)	38.67	40.10	49.28	64.38
Vdss/F (L)	157.36	162.31	172.50	191.08
Vdss/F, free (L)[a]	267.33	266.72	269.53	264.81

[a]Did not differ significantly between doses. All other parameters differed significantly between doses. (Duncan test, $P<0.06$)

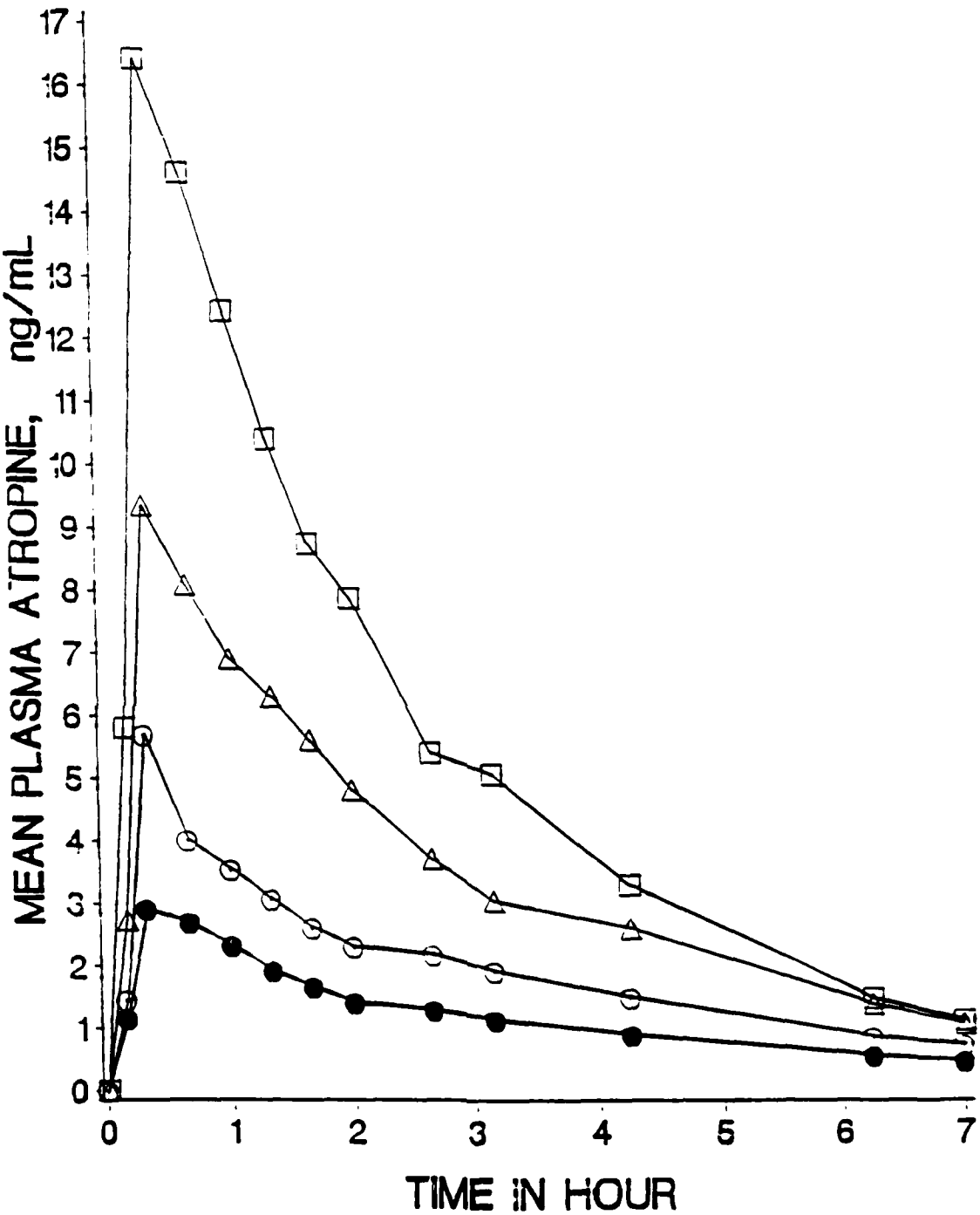

Figure 6. Mean plasma atropine concentration following intramuscular injection of 0.5 mg (solid circle), 1.0 mg (open circle), 2.0 mg (triangle) and 4.0 mg (square) of atropine. Each point represents the predicted value based on the pharmacokinetic values in Table I.

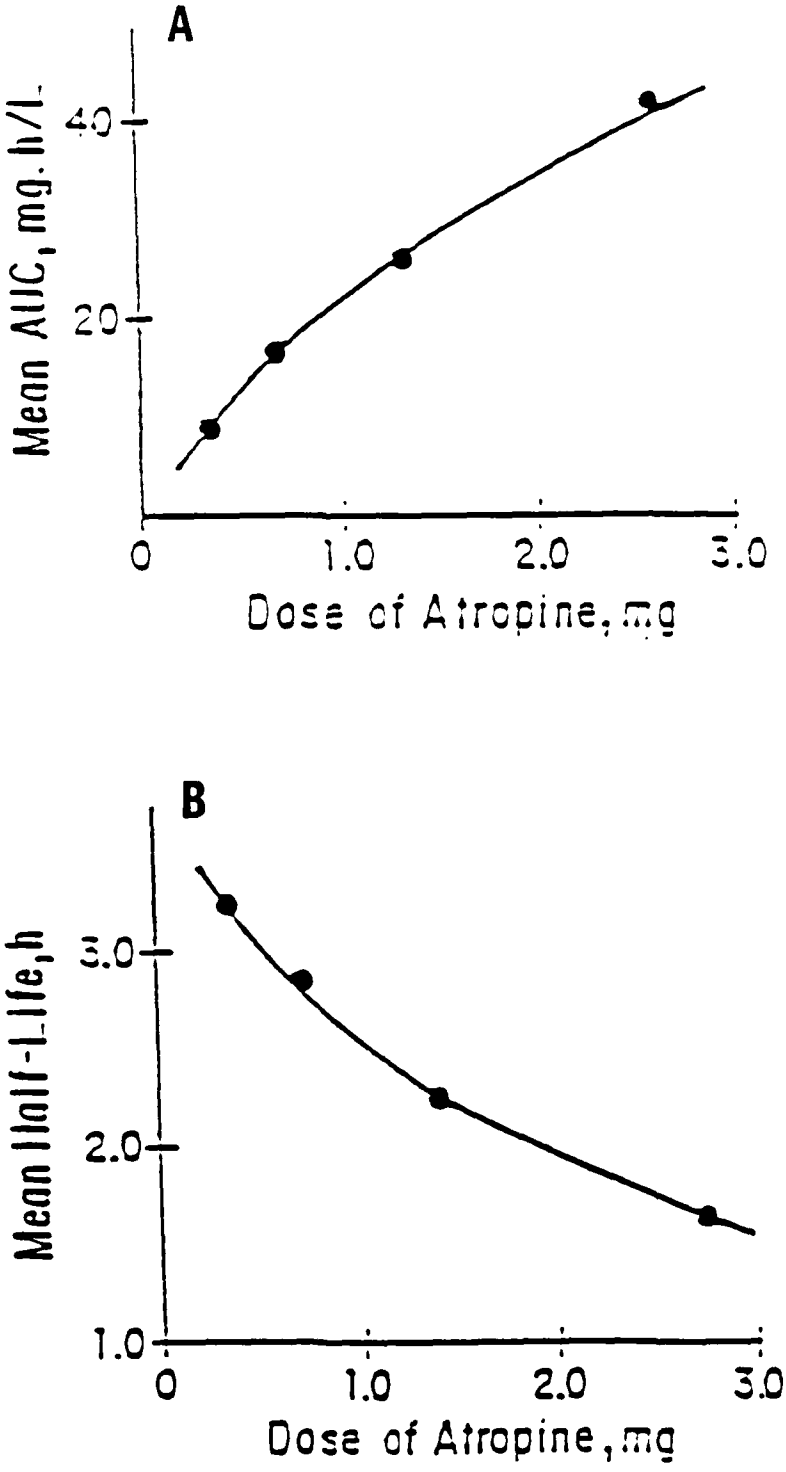

Figure 7. Relationship of atropine dose to mean area under the plasma atropine concentration vs. time curve (A) and to mean plasma atropine elimination half-life (B). The best-fit equation for Figure 2A is y=0.23x0.65; r=0.98. The best-fit equation for Figure 2B is y=3.4e-0.27; r=0.96.

concentration-dependent and non-linear both in vitro and in vivo (Table III). Atropine's low affinity for albumin binding sites but high affinity for AAG binding site was further demonstrated in human serum albumin solution (40 gm/L) where binding was about 2% yet binding was 42% to the alpha-1-acid glycoprotein solution (0.7 gm/L), under similar conditions (in vitro study). The dose-dependent (non-linear) binding may in part explain the decrease in elimination half-life as a function of dose.

Concentration-Effect Relationship

The hysteresis curves in Figs. 8 and 9 depict the time-dependent relationship between the mean atropine plasma concentration and mean scores for the hard SCT and DSS tasks and heart rate. A marked counterclockwise hysteresis was observed for the two performance measures and remarkably similar profiles were found for the different doses. In spite of a rapid rise in atropine levels during the initial 15 min following drug administration, the subjects showed very little change in behavioral performance. Substantial impairment was not evident until the drug concentration was either at the maximum level or had started to decrease.

The greatest discrepancy between drug concentration and effect was demonstrated by the DSS hysteresis curve for the 4.0 mg dose; specifically, DSS performance was still near maximum impairment ($p<0.003$) even when plasma atropine was barely detectable at the end of the test session (Fig. 9). While subjects eventually recovered from the drug effect on the hard SCT task, their SCT scores were initially impaired and continued to be impaired significantly ($p<0.003$) during the effect offset phase by the two higher doses in the presence of a more rapid decline of the atropine level (Fig. 8). SCT performance did not improve and return close to predrug values until after a considerable reduction of the atropine concentration. The hysteresis curves for the easy version of SCT task (not shown) were similar to those of the hard SCT task, indicating that task difficulty was not a major determinant of the offset rate for the drug effect.

In general, the curves representing the relationship between the physiological heart rate measure and atropine concentration were characterized by a collapsed hysteresis (Fig. 10). Thus, there was no lag between the time course for changes in plasma concentration and heart rate. As shown in Table IV, the results of the regression analyses provide additional evidence of a closer correlation between alterations in drug concentration and the atropine effect on heart rate. Substantially greater R^2 values were obtained for heart rate than for either the SCT or DSS tasks, particularly for 4 mg of atropine.

These differences between brain and peripheral atrial cardiac measures in the pharmacokinetic-pharmacodynamic relationship was analyzed further using the equation:

$$E = (E_{MAX} \cdot C_e^N) / (EC^N_{50} + C_e^N)$$

Where E is the predicted effect, E_{MAX} is the maximum change in predicted effect which can be produced by the drug, Ce is the time course of drug concentration at the effect site, and 'N' (Gamma) is the Hill coefficient.

Table III. Comparison of Plasma Unbound Fractions of Atropine Between In Vitro and In Vivo Studies

Studies	Plasma Atropine Concentration (ug/L)			
	2	5	10	20
In Vitro	0.56	0.61	0.65	0.71
In Vivo	0.55	0.62	0.63	0.73

Table IV. Proportion (R^2) of Task and Heart Rate Variance Predicted by Atropine Plasma Concentration[a]

Measure	Atropine Dose		
	1 mg	2 mg	4 mg
Entire Time Course			
Easy SCT	.01	.02	NS
Hard SCT	.01	.02	NS
DSS	NS	NS	NS
Heart Rate	.19	.09	.56
Effect Onset Phase[b]			
Easy SCT	NS	NS	NS
Hard SCT	NS	.03	.06
DSS	NS	.05	NS
Effect Offset Phase			
Easy SCT	.04	.05	.06
Hard SCT	.06	.07	.09
DSS	NS	.02	NS
Heart Rate	.27	.09	.55

[a]All R^2 values are significant at the $p<0.05$ level
[b]NS = nonsignificant

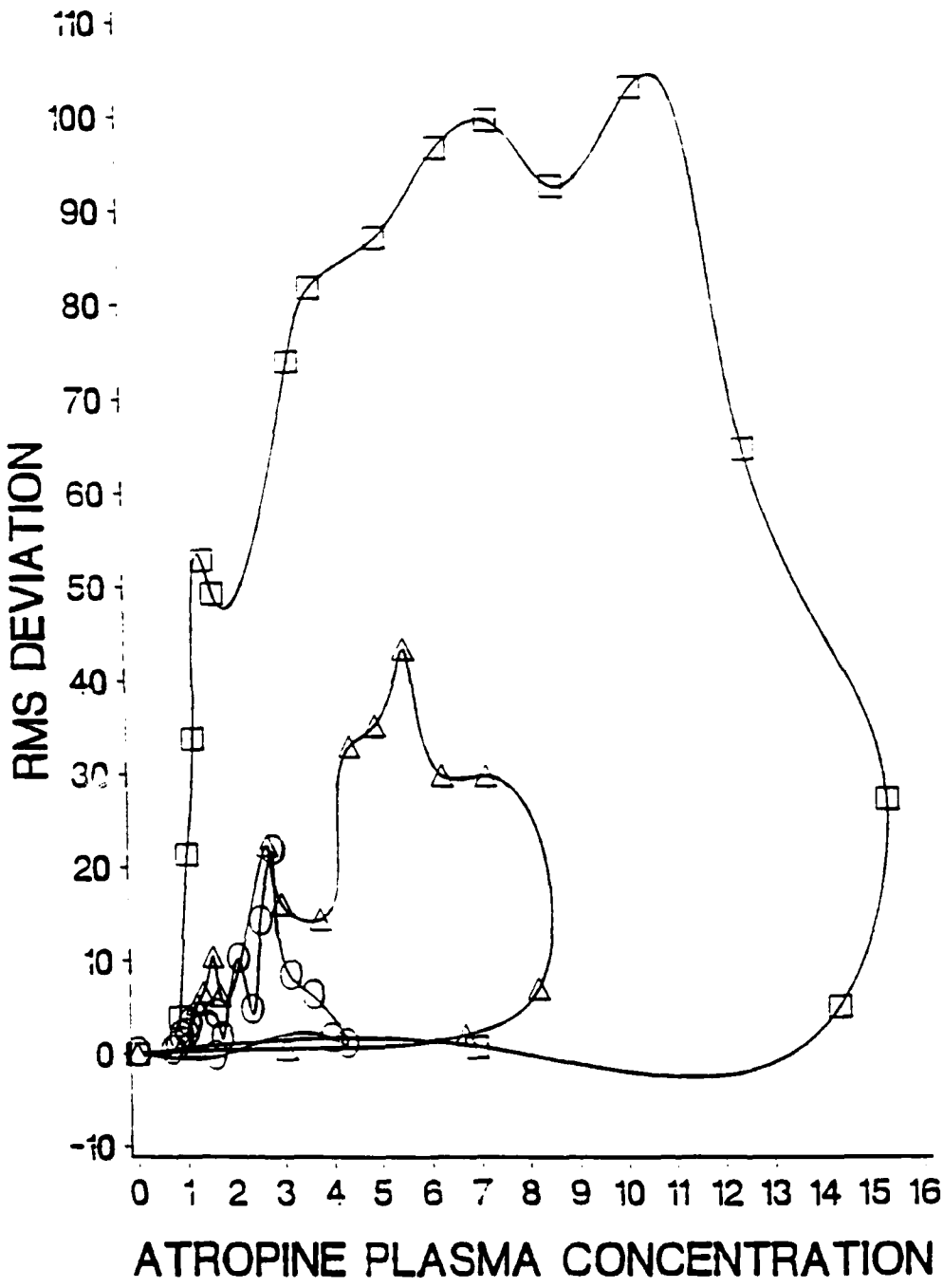

Figure 8. Performance on the hard difficulty level of the SCT task vs. atropine plasma concentration for the 1.0 mg (circle), 2.0 mg (triangle) and 4.0 mg (square) doses of atropine. Performance impairment is represented as mean number of unit changes from the predrug score (mean delta scores). The unit of measurement (RMS deviation) is described in the text. A smooth spline fitting procedure was used to draw the curve which connects consecutive testing time points for the assessment of behavior. Arrows denote the sequence of the observations.

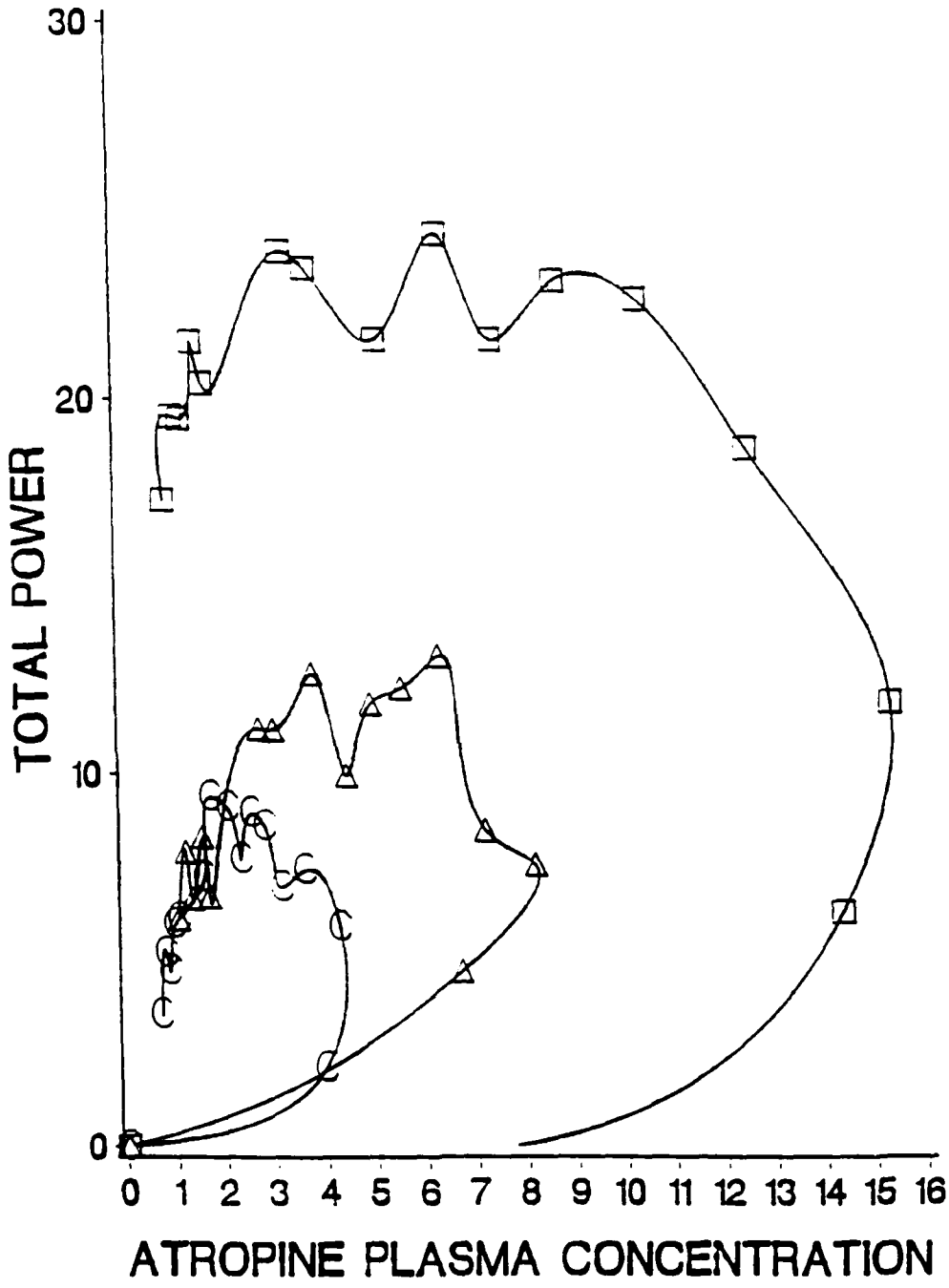

Figure 9. Performance on the DSS task vs. atropine plasma concentration for the 1.0 mg (circle), 2.0 mg (triangle) and 4.0 mg (square) doses of atropine. Performance impairment is expressed as mean delta scores. Power and delta scores, curves and arrows are described in Fig. 3.

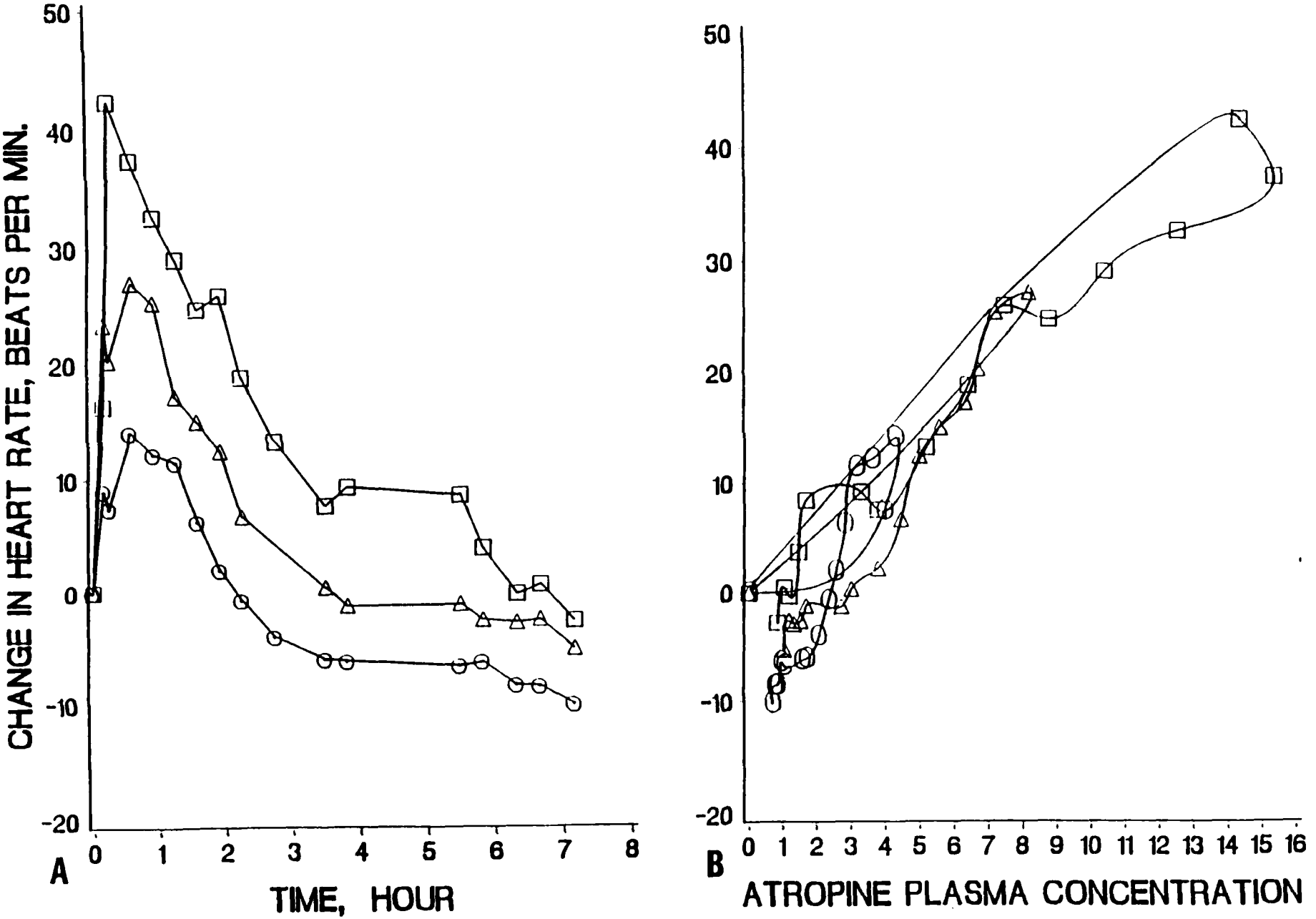

Figure 10. Mean increase in heart rate vs. atropine plasma concentration for the 1.0 mg (circle), 2.0 mg (triangle) and 4.0 mg (square) doses of atropine. Heart rate is expressed as mean delta scores. The unit of measurement, delta scores, curves and arrows are explained in Fig. 3.

In Table V, the lag time between plasma drug concentration changes for the 4.0 mg dose and effect change over time are noted in the $t_{\frac{1}{2}}k_{eo}$ values. The cognitive-neuromotor measures have substantial lag ($t_{\frac{1}{2}}k_{eo}$ values) whereas heart rate has no lag. These analyses assume that the temporal discrepancy is based on rate of exit and entry of drug to the effect compartment.

Growth Hormone Changes

To explore the concentration-effect relationship for GH, time course (Fig. 11A) and hysteresis, curves (Figs. 11B and 11C) were plotted for the four atropine doses. The GH results for the subgroup of eight subjects demonstrate a time course of responsivity that is very similar to the onset and offset of the atropine plasma levels. The GH t_{max} (time to reach maximum level) of 40 min lagged slightly the atropine t_{max} at 20 min. The collapsed hysteresis curves for GH (Figs. 11B and 11C) are quite similar to the profile for the peripheral antimuscarinic effect on heart rate (Fig. 10).

Discussion

As discussed in the Introduction, the present experiments were designed to examine the relationship between the plasma drug concentration and pharmacological effects of atropine. An important issue concerns the question of whether this relationship and its underlying mechanisms are similar for the peripheral and central nervous systems. In our studies the central effects were assessed by both the cognitive DSS and neuromotor SCT tasks and the peripheral effects by heart rate and GH.

The atropine concentration vs. time profiles for the different doses are best described by a two-compartment model with first-order absorption and lag time. Atropine is absorbed rapidly following intramuscular injection, as indicated by the short absorption half-life (Table II) and t_{max} for all four doses (Fig. 6). The t_{max} obtained in the present studies is comparable to values found in the literature for similar dosages (Berghem et al., 1980; Gundert-Remy et al., 1980). As expected for the intramuscular route of administration, the absorption half-life was constant across doses. On the other hand, the elimination half-life decreased with increasing dose from 3.30 hr to 1.62 hr (Table II) and corresponded to the values reported by others (Virtanen et al., 1980; Hayden et al., 1979; Hinderling et al., 1985b). Similarly, the clearance of atropine ranged from 38.67 to 64.83 L/hr and was greater for the higher doses. The large volume of distribution is indicative of significant tissue uptake. For several pharmacokinetic parameters the data suggest a non-linear relationship across the four doses used.

Comparison of the pharmacokinetic data to the heart rate results indicates a close relationship between plasma atropine levels and concurrent changes in heart rate, particularly for the highest dose. A collapsed hysteresis was evident in the curves depicting the relationship between drug concentration and this physiological variable (Fig. 10). At 4 mg the observed t_{max} (time to maximum impairment) for heart rate corresponded to the t_{max} of the atropine C_{max}. For the lower doses, heart rate t_{max} was only slightly longer than the atropine t_{max}. For all four doses, the rate of the return of heart rate to predrug levels approximated the rate of elimination of atropine

Table V. Pharmacodynamic Parameters of Atropine after 4 mg IM Dosing.

Effects	Parameters			
	$t_{1/2}k_{eo}$[1] (hr)	EC_{50} (ng/mL)	Gamma (N)	E_{max}
(DSS)[2] ($\triangle$ Power)	1.26	7.78	0.5	48.6
(SCT)[3] ($\triangle$ RMS Deviation)	0.67	7.25	1.7	167.9
Heart Rate ($\triangle$)	----	16.70	1.19	81.3
Growth Hormone ($\triangle$%)	0.15	10.75	1.23	124.9

[1]Equilibration Time-Lag Between Plasma and Effect Compartment
[2]Digit Symbol Substitution
[3]Subcritical Tracking Effect

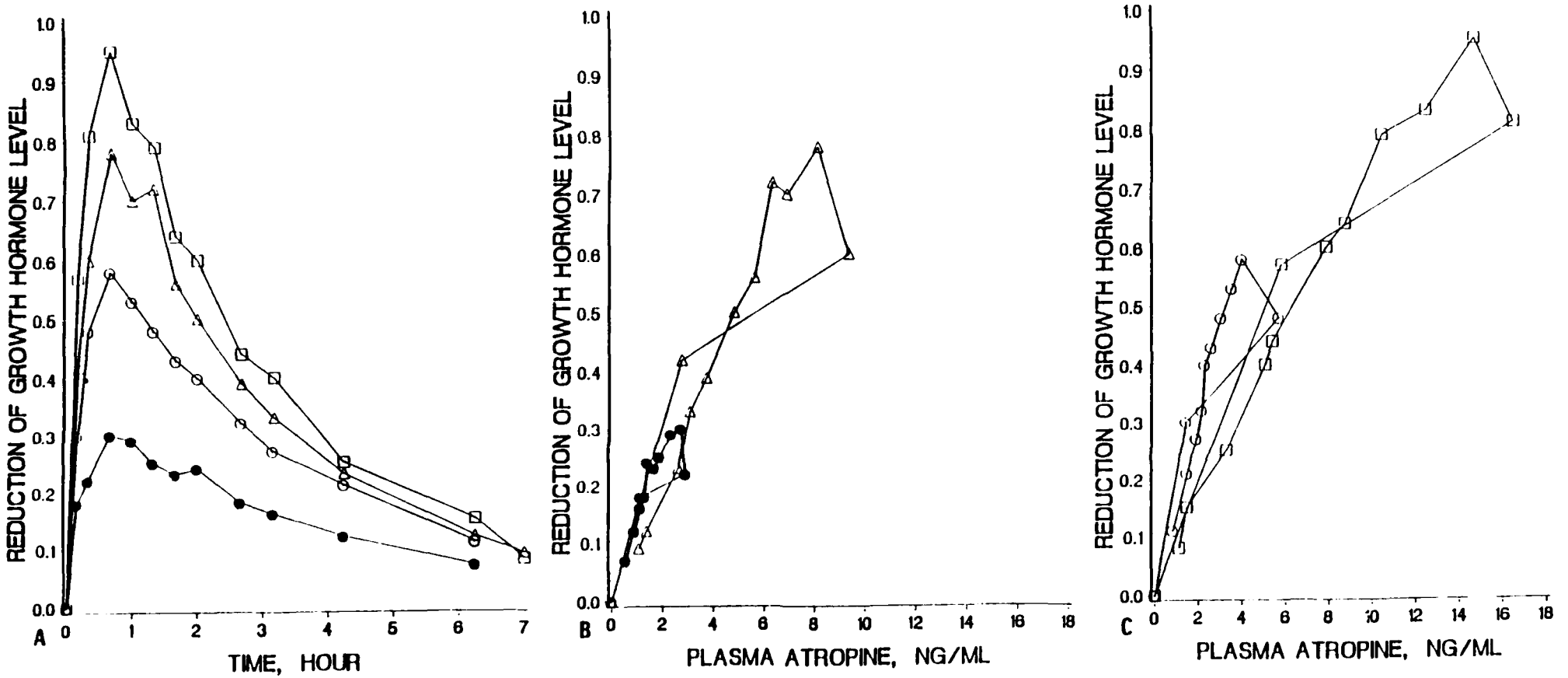

Figure 11. Reduction of growth hormone levels for 8 subjects following intramuscular injection of 0.5 mg (solid circle), 1.0 mg (open circle), 2.0 mg (triangle) and 4.0 mg (square) doses of atropine. Reduction of growth hormone level is calculated using the following equation: Eo-E/Eo, where Eo = growth hormone level at predrug, E = growth hormone level at each pre- and post-drug assessment time point.

from plasma. Thus, for a single dose of atropine the rate of return of heart rate to normal values provides a rough clinical estimate of declining atropine plasma level, although this relationship has not been verified in the presence of a cholinesterase inhibitor.

The similarity between the rate of change in heart rate and drug plasma levels during the effect offset phase indicates that pharmacokinetics is the major rate-limiting contributor to the cardiac effect offset. For the 1.0 and 2.0 mg dose the heart rate effect offset was over during the fast elimination phase whereas the 4.0 mg offset extended into the slow elimination phase. The heart effect offset rate for the 1.0 and 2.0 mg dose was 0.4 hr, similar to the atropine $t_{\frac{1}{2}}\alpha$, whereas the 4.0 mg dose offset (1.2 hr) reflects a hybrid of $t_{\frac{1}{2}}\beta$ and $t_{\frac{1}{2}}\alpha$.

Unlike the heart rate effect, recovery from DSS impairment is barely perceptible during the 7 hr of monitoring (Fig. 9). These discrepancies between plasma pharmacokinetics can be analyzed with PK-PD models (see Table II) that provide evidence of extensively slow exit of drug from the effect compartment under the assumptions of this analysis. However, the lag may also reflect either a relatively irreversible binding or a transduction induced long-term effect (e.g., breakdown of inositol lipids and mobilization of cellular calcium) (Berridge and Irvine, 1984). Certainly the extent of prolonged response is not secondary to pharmacokinetic parameters. The long-lasting DSS effect is a stark contrast to the collapsed hysteresis curve for heart rate as well as the heart rate offset half-life that approximates atropine elimination half-life (Table II). On the other hand, the profile for SCT impairment offset falls somewhere in between the DSS and heart rate effects and has an estimated offset half-life of approximately 3 hrs. and a pronounced counterclockwise hysteresis curve (Fig. 8).

These results are consistent with the hypothesis that muscarinic receptor heterogeneity and kinetics are major contributors to the differences between the time course of the atropine effect on heart rate and the memory-psychomotor speed task (DSS). Specifically, the slower onset and more prolonged duration of the DSS impairment may be regulated by the high M_1 receptor density in hippocampus, cerebral cortex and corpus striatum (Watson et al., 1982; Watson et al., 1983), while the faster onset and recovery of the heart rate effect may be due to the predominant M_2 sites in the cardiac atrium (Wess et al., 1984; Brown, 1980). The SCT impairment may reflect contributions of components of extrapyramidal and cerebellar functions, which have different distributions of "M_1/M_2 receptor types" and responsivity of phosphoinositide turnover (Fisher and Barbus, 1985). If these hypothetical relationships are confirmed in future research, then prediction of duration of action in humans for new selective M_1 and M_2 antagonists should be possible.

GH inhibition was not a part of our original set of hypotheses since the M_1, M_2 receptor type regulating GH inhibition is not known. Based on the finding that the time course of the growth hormone inhibition was very similar to that of heart rate, the effect of atropine on GH appears to be primarily mediated by a M_2 receptor type. However, the hypothesis regarding the relation of the time course for the growth hormone effect to M_1/M_2 receptors is open to critical experimentation with specific M_1/M_2 blockers.

The following results of the present studies might be considered in the utilization of atropine for prophylaxis of anticholinesterase poisoning, especially the IM 2 mg formulation given to military personnel. First, the impairment profiles for the cognitive and neuromotor tasks can be used as general guidelines for the magnitude and duration of CNS impairment that may be expected following 1.0, 2.0, and 4.0 mg of atropine. Second, this information may be especially useful in training medical personnel on how to estimate when the functioning ability of military personnel will return to normal. Third, the lack of correspondence between the time courses of the pharmacokinetics and CNS effects of atropine is particularly noteworthy. Finally, the preferential binding of atropine to AAG may be important in the clinical setting. Since AAG levels may be elevated by physiological stress, trauma or other conditions and result in higher protein binding and less free atropine in plasma, individuals in a stressful situation may not have the same magnitude and time course of effect by the same dose of as individuals who are not experiencing any stress. Considering the evidence of the dose-dependent nature of protein binding, this elevation of AAG levels would appear to be a more important factor as dose is increased. The potential contribution of the concentration-dependent alteration of protein binding to the drug effect is suggested by the greater rise in the magnitude of the maximum impairment of the SCT and DSS tasks for the higher doses. However, heart rate and GH demonstrated a non-linear increment in the opposite direction from that expected by increasing the free fraction. A likely explanation is that the heart rate and GH were at asymptomatic levels for the higher doses.

In conclusion, the results of the present study are consistent with findings from basic science laboratories and support the hypotheses of marked differences in peripheral M_2 vs. CNS M_1 muscarinic receptors as well as differences between brain regions underlying the SCT vs. DSS tasks. In addition, the data indicate that differential receptor distribution is a pharmacologically relevant factor, especially in the determination of the differential time course for the CNS and peripheral effects of atropine.

Summary

Two experiments were designed to examine the pharmacokinetic-pharmacodynamic relationship for the CNS and peripheral effects of atropine. According to a random Latin square design, healthy young male volunteers were given intramuscular injections containing single doses of placebo or 0.5, 1.0, 2.0 or 4.0 mg of atropine. The CNS tests included wheel tracking, a coordination task, and digit symbol substitution, a memory-psychomotor speed task, and the physiological variable was heart rate. Several connections between the atropine effects and muscarinic (M_1 and M_2) receptor activity were hypothesized; that is, the involvement of peripheral M_2 receptors in the heart rate effect; of M_1 receptors in the memory-psychomotor effect and of both receptors in the coordination effect. The pharmacokinetics of atropine were best described by a two-compartment model with very rapid first order absorption. For all four doses changes in plasma atropine levels and heart rate closely overlapped throughout most of the time course. In contrast, the differential time course of changes in atropine levels and behavioral impairment indicates that pharmacokinetics is not the primary rate-limiting mechanism for the CNS effects of atropine. The stark differences in the

concentration effect relationships of the CNS and cardiac muscarinic effects are discussed in relation to known M_1/M_2 receptor distribution and pharmacodynamics.

List of Abbreviations:

ANOVA = analysis of variance
AUC = area under the plasma vs. time curve
CLp/F = total plasma clearance
CNS = central nervous system
DSS = digit symbol substitution
GH = growth hormone
IM = intramuscularly
MRT = mean residence time
NC = number of correct responses
RMS = root mean squared
RT = reaction time
SCT = subcritical tracking
$t_{\frac{1}{2}}$ = half-life
Vdss/F = total volume of distribution at steady state
WAIS-R = Wechsler Adult Intelligence-Revised

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Part III: Utilization of Test Batteries Developed with Atropine

Introduction

Practical Considerations

The concerns of military medical units about atropine dosing for real or anticipated cholinesterase poisoning involve several of the following questions: 1) what cognitive-neuromotor skills will be impaired and for how long after a given dosage, 2) what are the dose response profiles for given skills, 3) in the field, what measures will help predict current levels of atropine plasma concentration, and 4) what measures are not good predictors of central nervous system (CNS) impairment time course, i.e., tasks inappropriate for a testing battery.

Behavioral and Physiological Measures Sensitive to Atropine

Overall, the subcritical tracking (coordination) task appears to have the most sensitive and concentration dependent profile for atropine impairment. The three-dimensional figures (Figs. 12 & 13) illustrate the combined time course and concentration dependent effects. Digit symbol substitution has a marked step up in effect from the 2.0 mg dose to the 4.0 mg dose. However, once the effect is produced, it has a sustained time course that is relatively independent of rapidly falling atropine plasma concentration (Figs. 14 & 15). These three-dimensional plots provide a reasonable estimate of the time course of atropine plasma concentration and impairment time course for doses given. Such plots could be used by those in the field for general estimates of return to function after atropine dosing in the absence of other toxins.

The faster offset for tasks involving coordination (i.e., tracking) than for tasks that have complex reaction time (psychomotor speed) components are confirmed in 1) the slow offset components in the digit-symbol memory task (Fig. 16); the continuous performance task (Fig. 17), and the keyboard reaction time task (Fig. 18),; 2) the comparative test requiring both tracking and continuous, simultaneous performance, i.e., divided attention task, clearly demonstrates the faster offset for tracking coordination (See Fig. 19).

From these tests, the conclusion is drawn that complex psychomotor speed tasks have a prolonged offset of impairment way beyond what would be expected from atropine blood levels. Unless necessary, someone who had injected 2.0 to 4.0 mg of atropine should not be required to engage in dangerous tasks requiring complex psychomotor speed for at least 6 to 10 hours.

A rough measure of atropine plasma level offset can be obtained by keeping track of the decline in atropine induced tachycardia. (See Fig. 20, and note the linear heart rate relation to plasma-atropine level) Obviously, the caveat that stress could also maintain tachycardia should be considered; but in the presence of baseline heart rate or bradycardia, one could

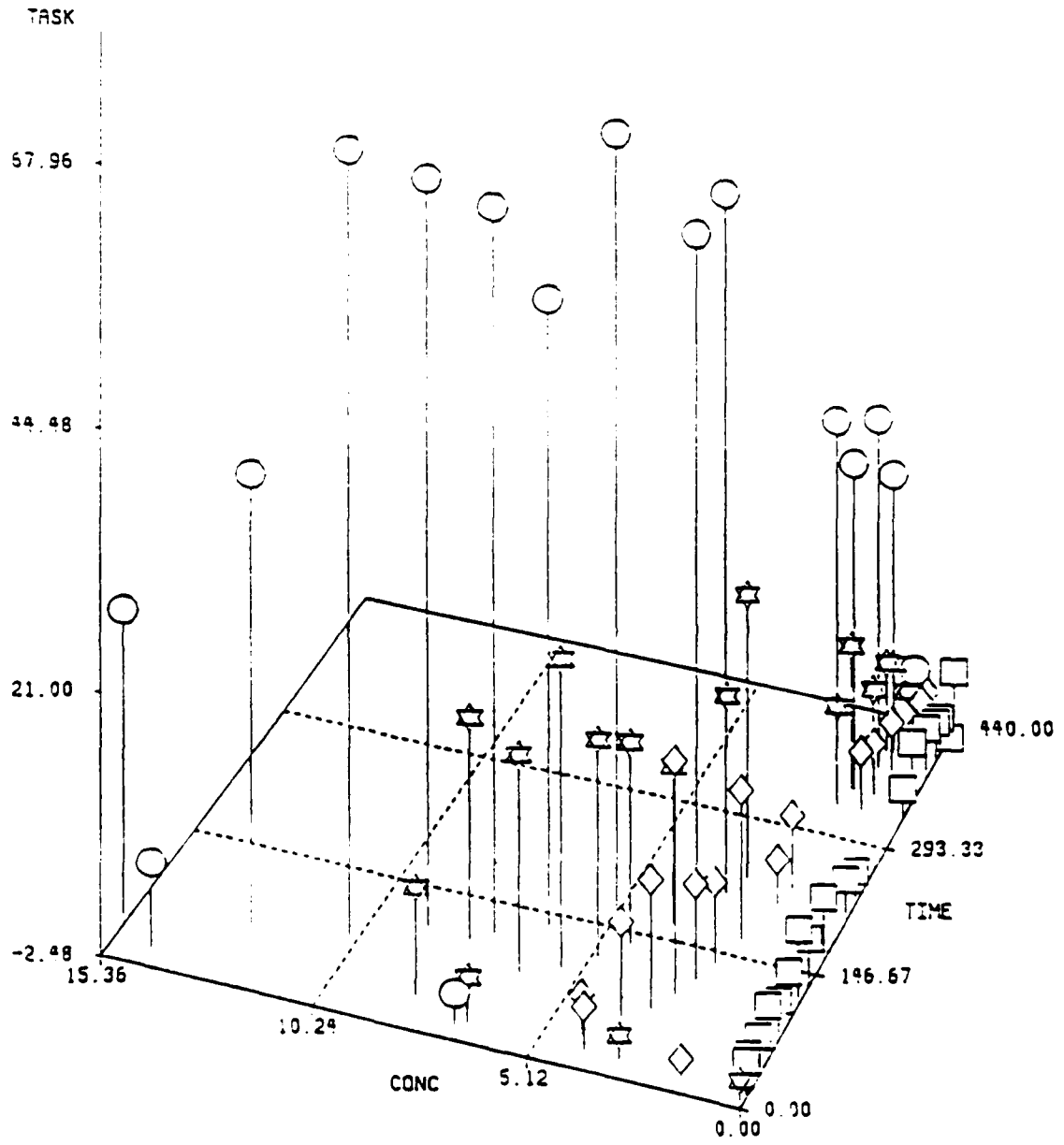

Figure 12. Three-dimensional scatter representation of the SCT task (vertical axis) as a function of atropine concentration and min post drug injection. Four doses of atropine, 0.0, 1.0, 2.0 and 4.0 mg, are represented as squares, diamonds, stars and circles, respectively. Each point represents a mean delta change scores for all subjects. The plot was done using the three-dimensional plotting procedure (G3D) of SAS. The delta score is explained in Figure 1. Atropine plasma values are the same as the mean values in Figure 14.

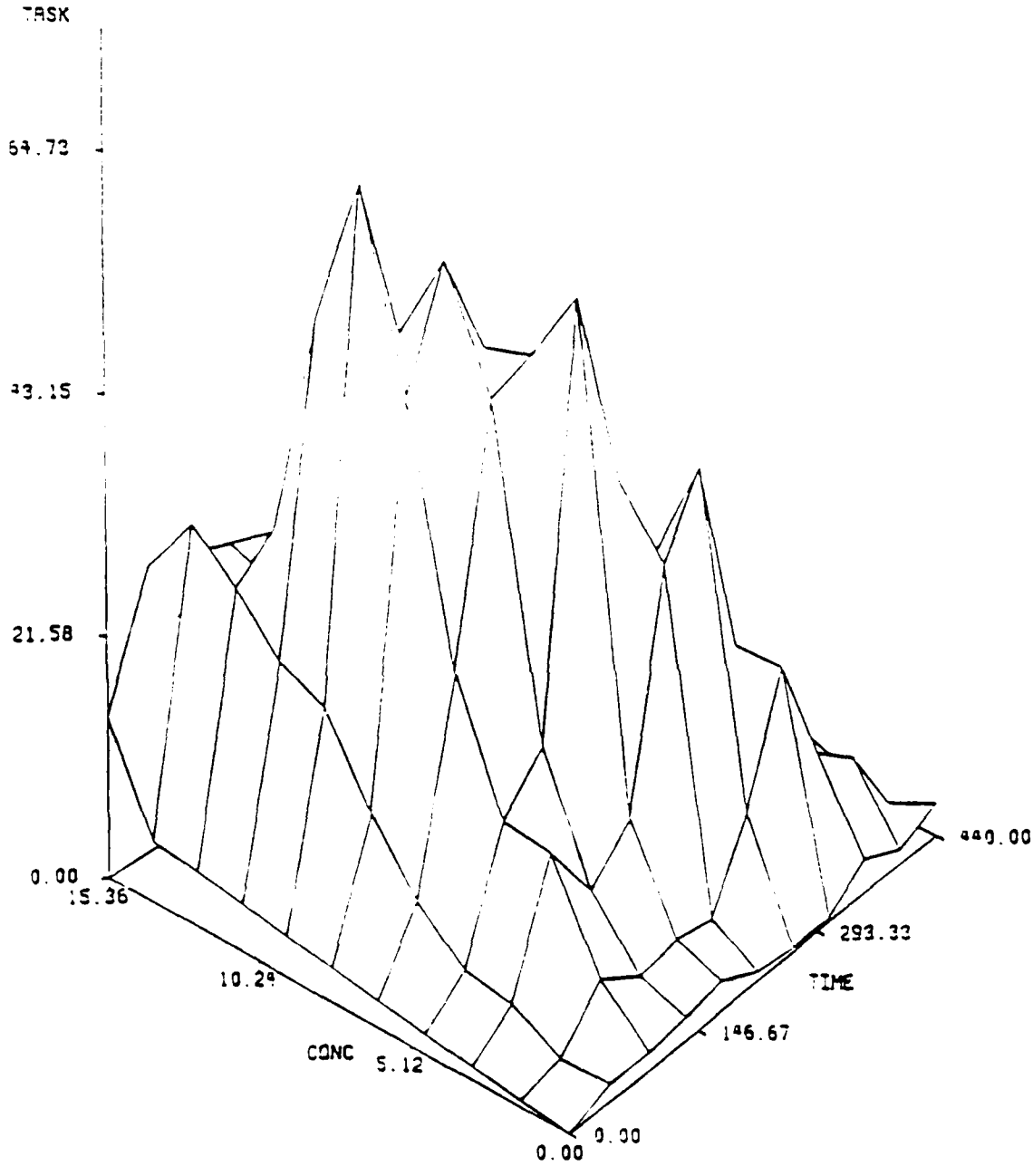

Figure 13. Three-dimensional surface representation of the SCT task (vertical axis) as a function of atropine concentration and min post drug injection. The surface was generated using the SAS grid procedure that does a linear interpolation within a set of triangular regions formed from the input data set. The surface is based on mean delta change scores for all subjects. The surface represents four doses of atropine, 0.0, 1.3, 2.0 and 4.0 mg. The delta score is explained in Figure 1. Atropine plasma values are the same as the mean values in Figure 14.

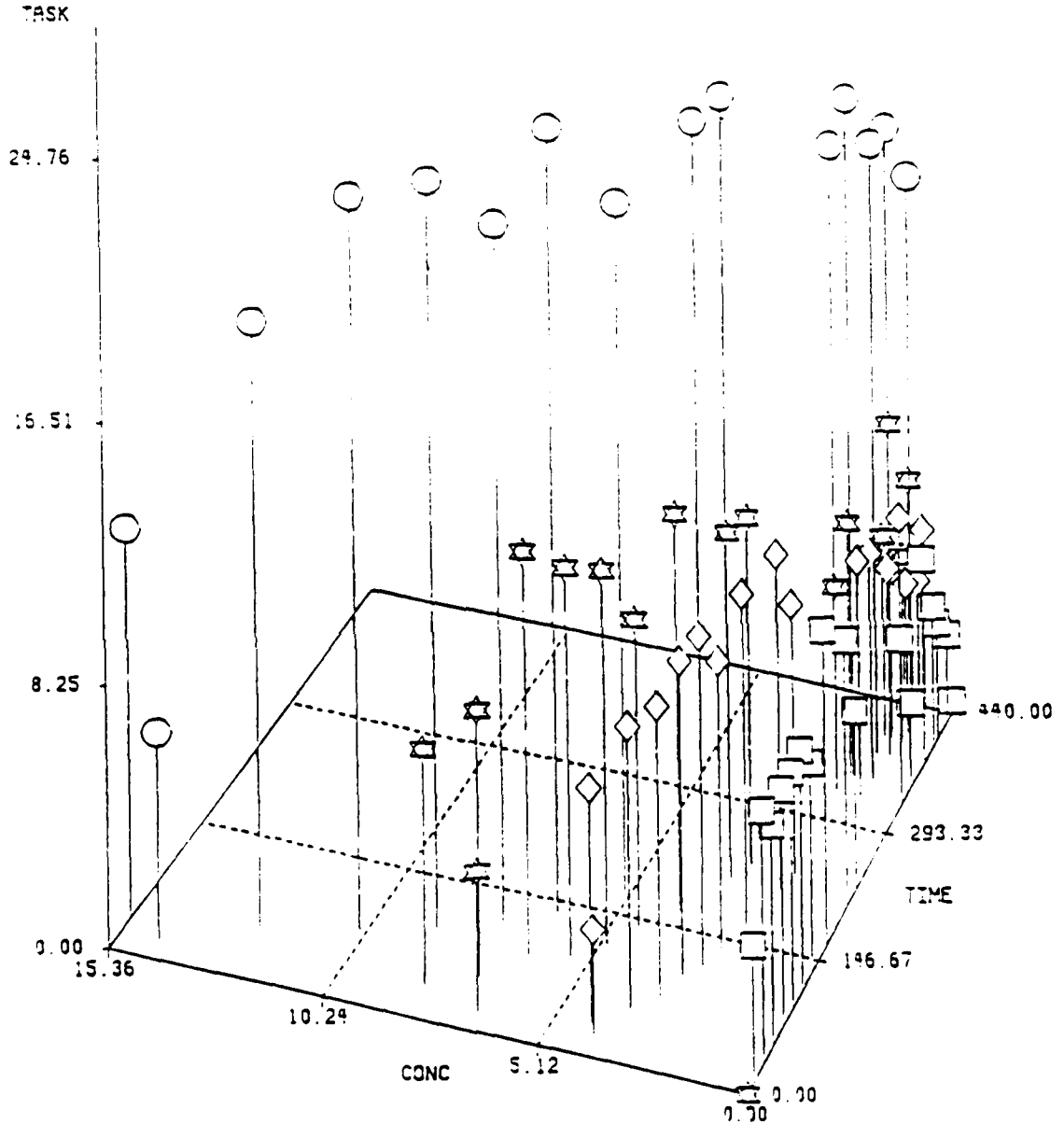

Figure 14. Three-dimensional scatter representation of the DSS task (vertical axis) as a function of atropine concentration and min post drug injection. Four doses of atropine, 0.0, 1.0, 2.0 and 4.0 mg, are represented as squares, diamonds, stars and circles, respectively. Each point represents a mean delta change scores for all subjects. The plot was done using the three-dimensional plotting procedure (G3D) of SAS. The delta score is explained in Figure 1. Atropine plasma values are the same as the mean values in Figure 14.

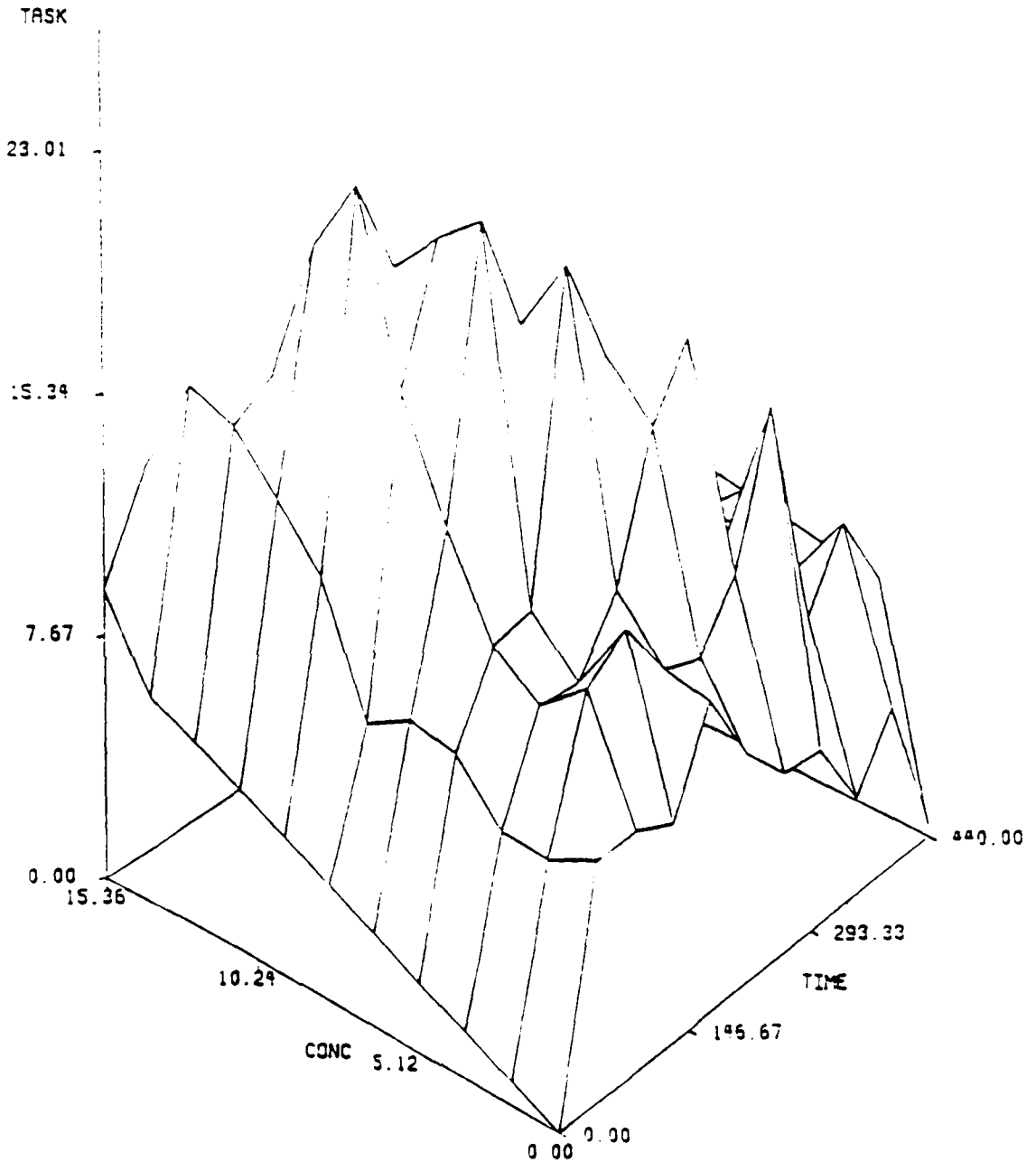

Figure 15. Three-dimensional surface representation of the DSS task (vertical axis) as a function of atropine concentration and min post drug injection. The surface was generated using the same procedure as in Figure 26. The delta scores and atropine plasma levels are described in Figure 26.

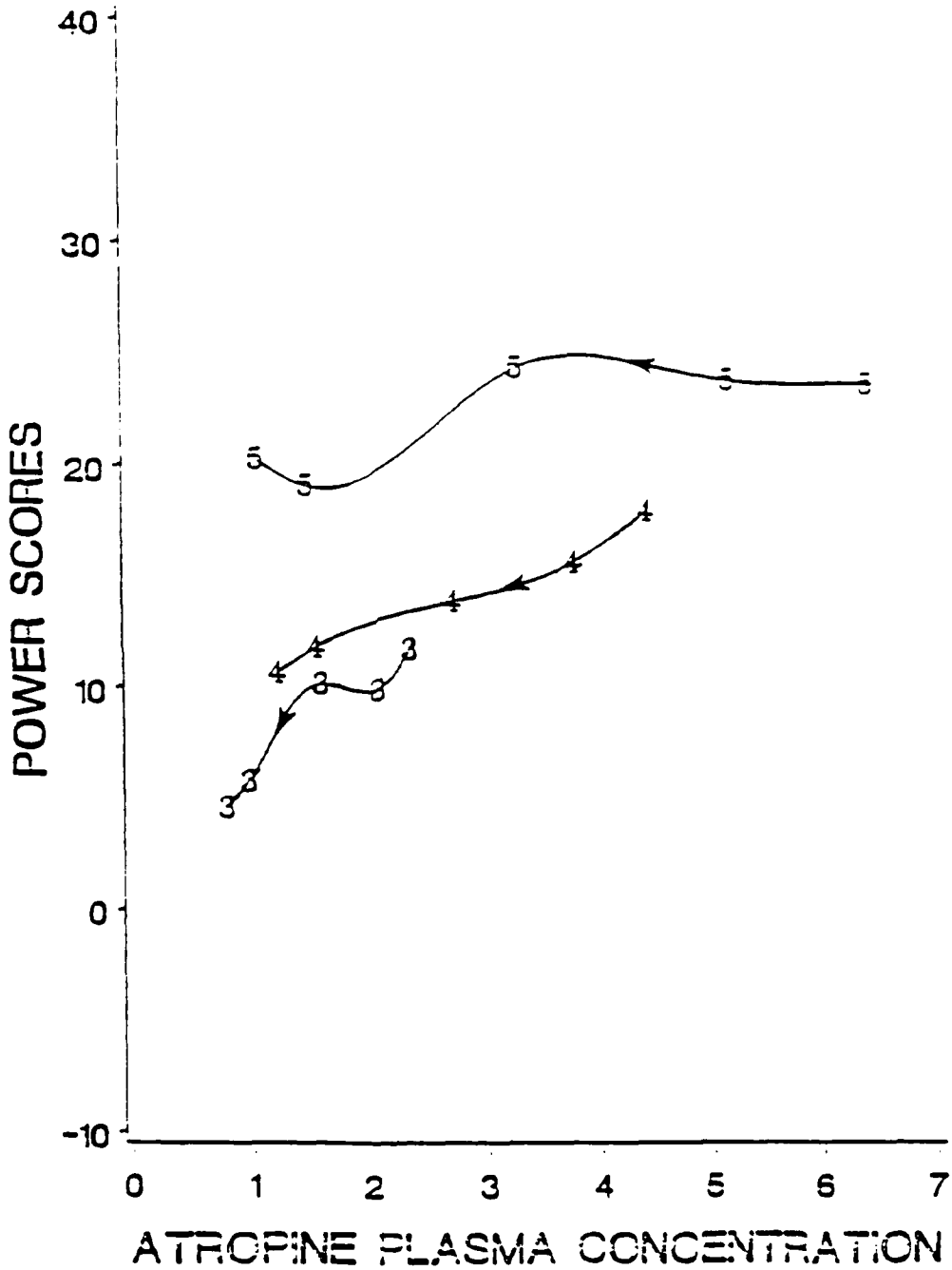

Figure 16. Performance on the digit symbol memory task vs. atropine plasma concentration for the 1.0 (3), 2.0 (4) and 4.0 (5) mg doses of atropine. Performance impairment is expressed as mean delta scores and is explained in Figure 7. Atropine plasma values, curves and arrows are the same as in Figure 16.

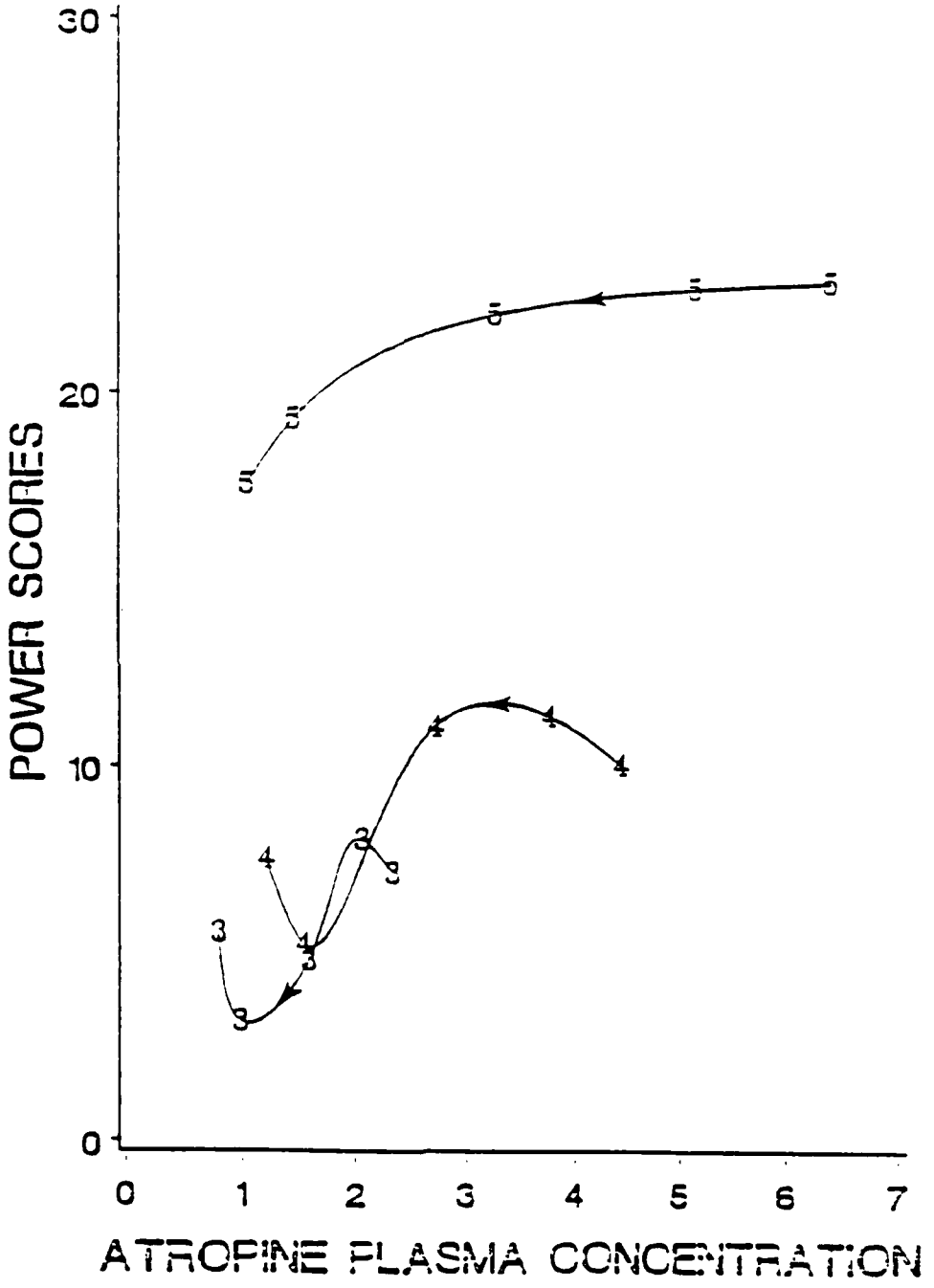

Figure 17. Performance on the continuous performance task vs. atropine plasma concentration for the 1.0 (3), 2.0 (4) and 4.0 (5) mg doses of atropine. Performance impairment is expressed as mean delta scores and is explained in Figure 4. Atropine plasma values, curves and arrows are described in Figure 16.

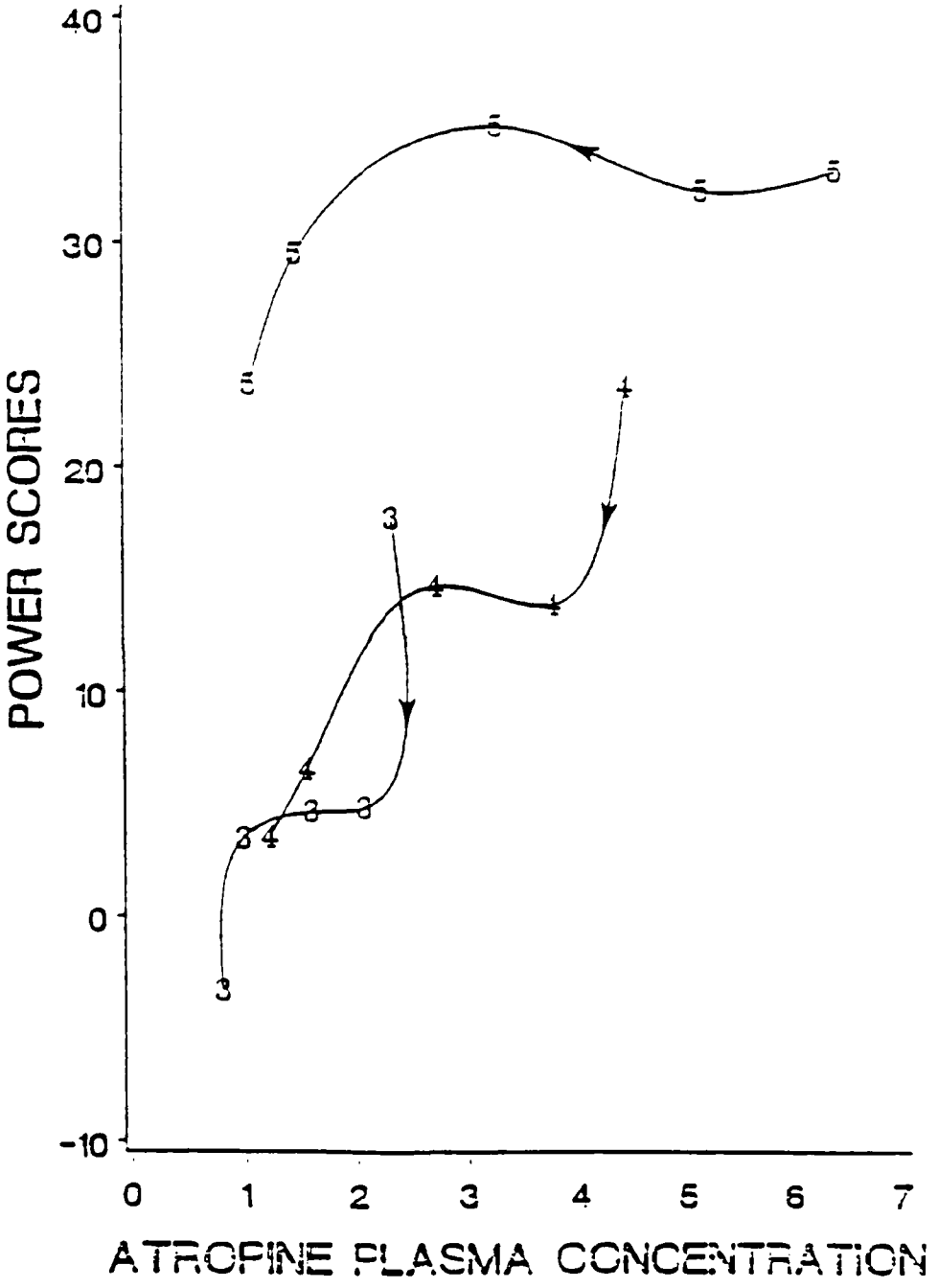

Figure 18. Performance on the digit-keypad reaction time task vs. atropine plasma concentration for the 1.0 (3), 2.0 (4) and 4.0 (5) mg doses of atropine. Performance impairment is expressed as mean delta scores and is explained in Figure 3. Atropine plasma values, curves and arrows are described in Figure 16.

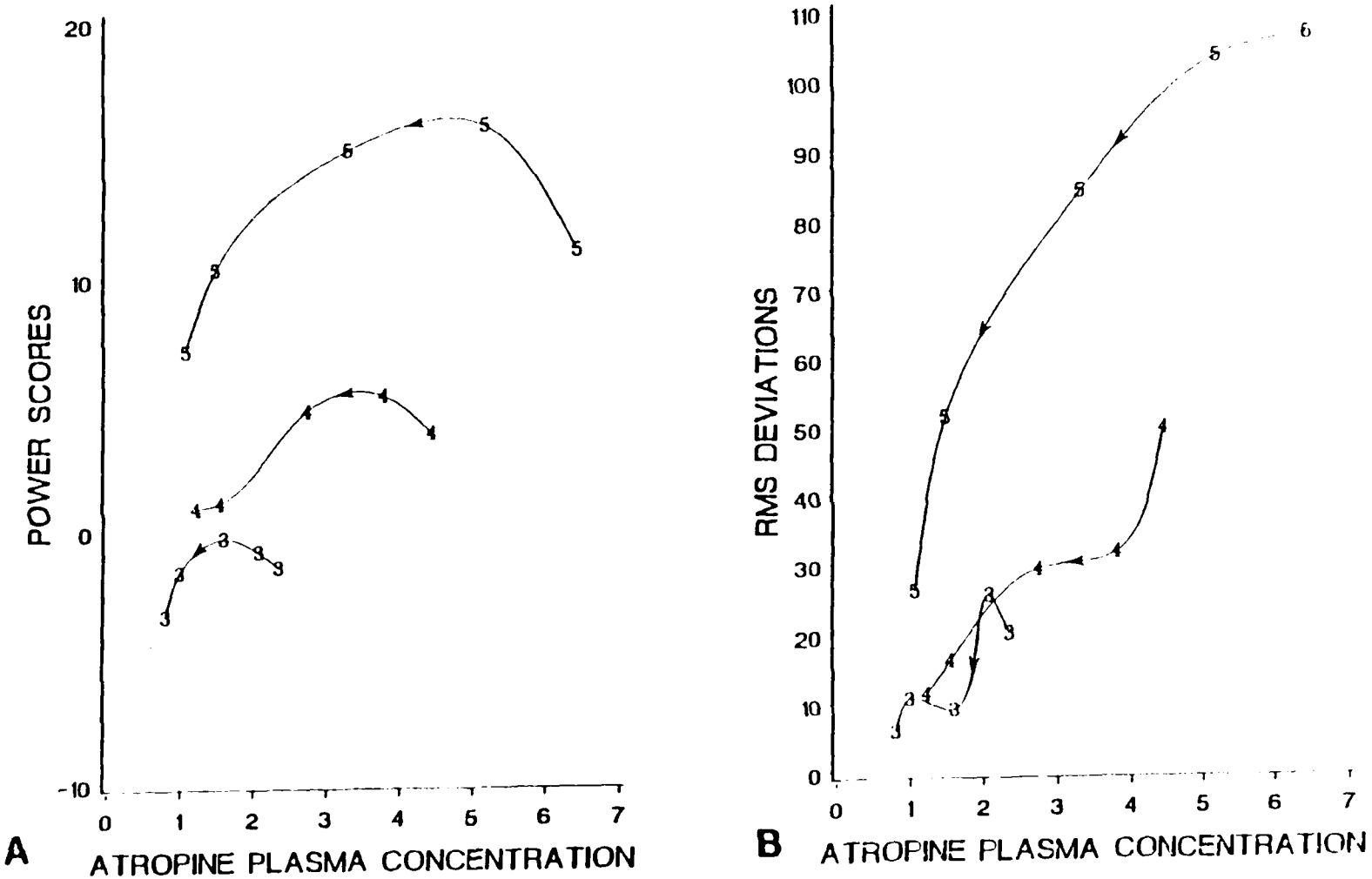

Figure 19. Performance on the divided attention task vs. atropine plasma concentration for the 1.0 (3), 2.0 (4) and 4.0(5) mg doses of atropine. Impairment on the continuous performance (20A) and hard difficulty level of the SCT (20B) portions of the task are shown. Performance impairment for each part of the divided attention task is expressed as mean delta scores and is explained in Figures 5 (continuous performance) and 6 (SCT). Atropine plasma values, curves and arrows are the same as in Figure 16.

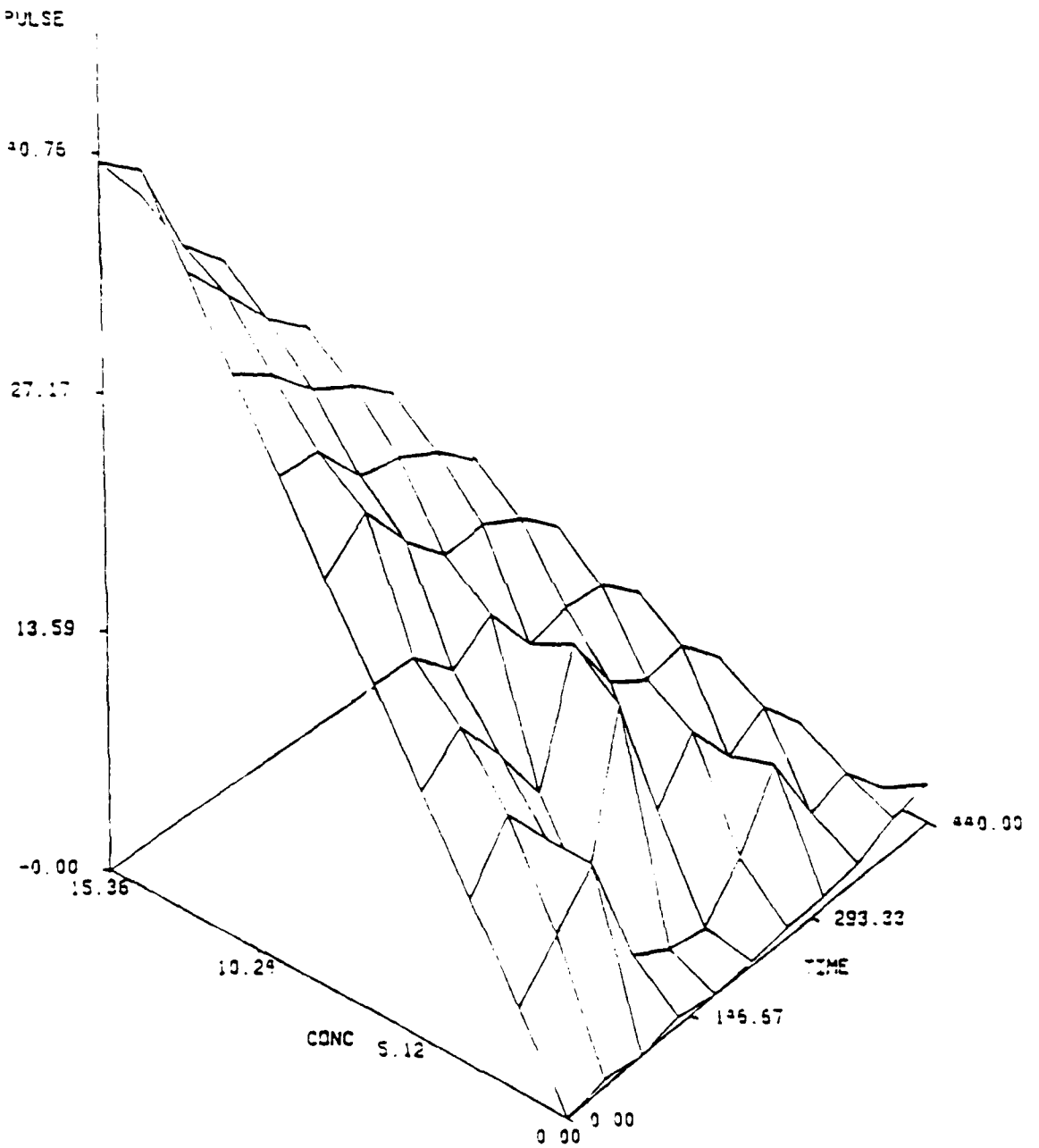

Figure 20. Three-dimensional surface representation of the increase in heart rate (vertical axis) as a function of atropine concentration and min post drug injection. The surface was generated using the same procedure as in Figure 26. The heart rate measure and atropine concentration levels are described in Figures 9 and 16, respectively.

reasonably assume that plasma atropine level is no longer protective against anti-cholinesterase poisoning for many peripheral effects.

Over the last year of the contract, considerable effort was made to develop new tasks that would be as sensitive to and have as good dose response to atropine as those found with the "anchor" task, subcritical tracking. None of the additional new development tasks met these criteria (See annual report for 1987-1988). Compared to the profiles for subcritical tracking, pendulum body sway and saccade eye task (Figs. 21, 22 and 24) were a poor second best. Both of the latter tests had only a high dose effect. Even worse at detecting impairment were rapid hand alternation task and rapid eye alternation task as well as the profile of dependent variables derived from the number-memory task. None of the tasks warrant further development for use with atropine impairment unless specific abilities need to be tested.

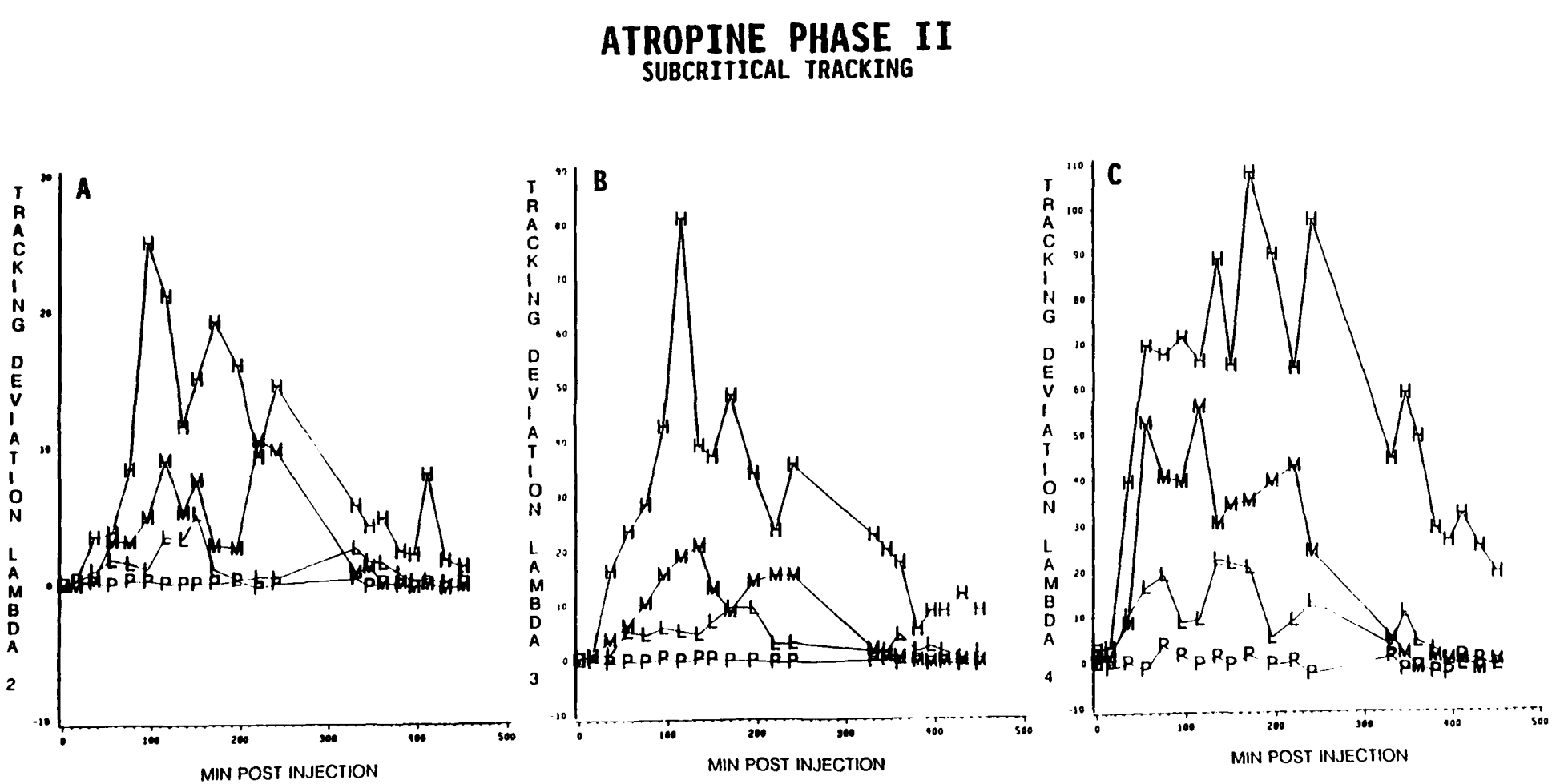

Figure 21. P = Placebo, L = 1.0 mg dose, M = 2.0 mg dose, and H = 4.0 mg dose.

ATROPINE PHASE II
PENDULUM SWAY TASK

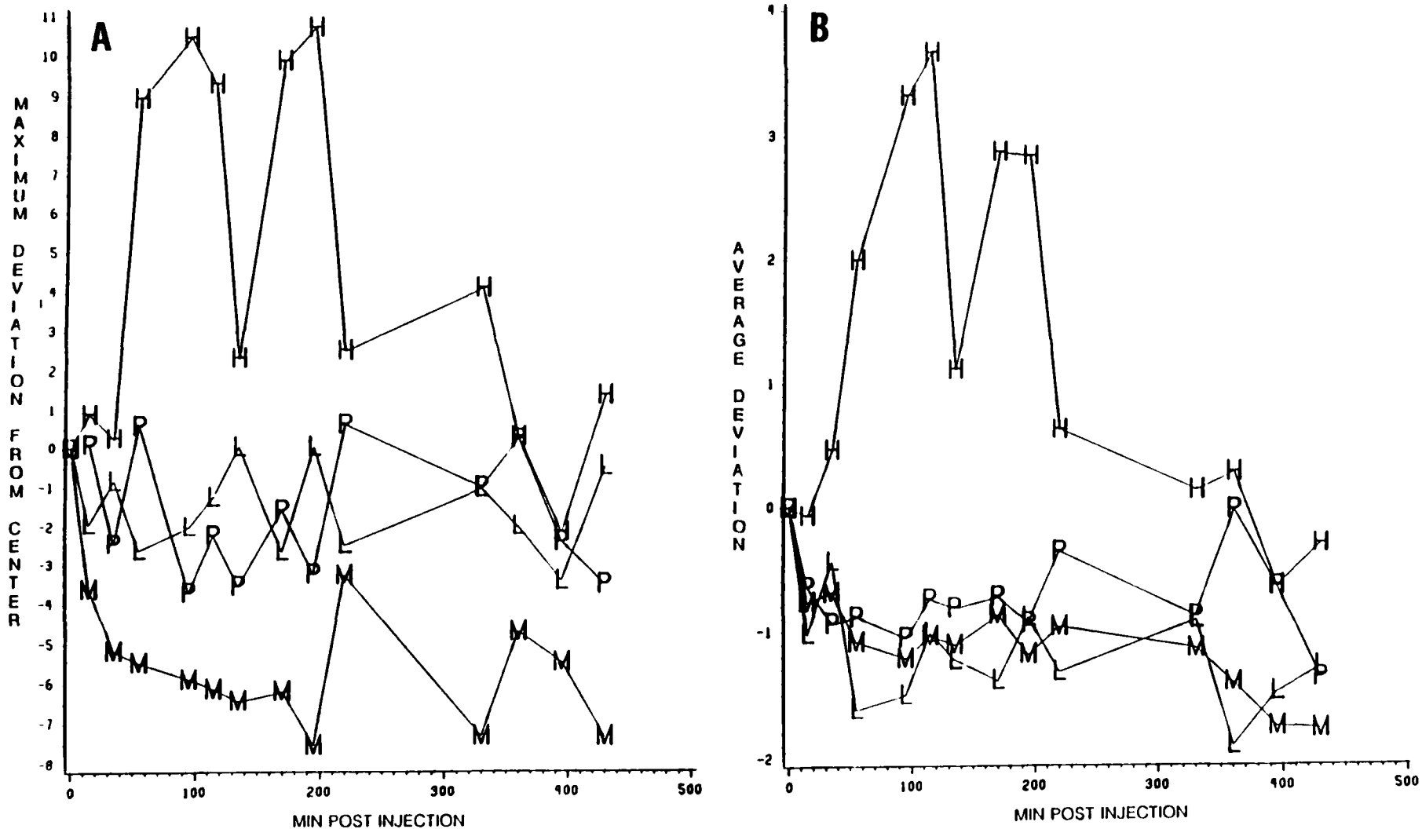

Figure 22. P = Placebo, L = 1.0 mg dose, M = 2.0 mg dose, and H = 4.0 mg dose.

ATROPINE PHASE II
HAND ALTERNATION TASK

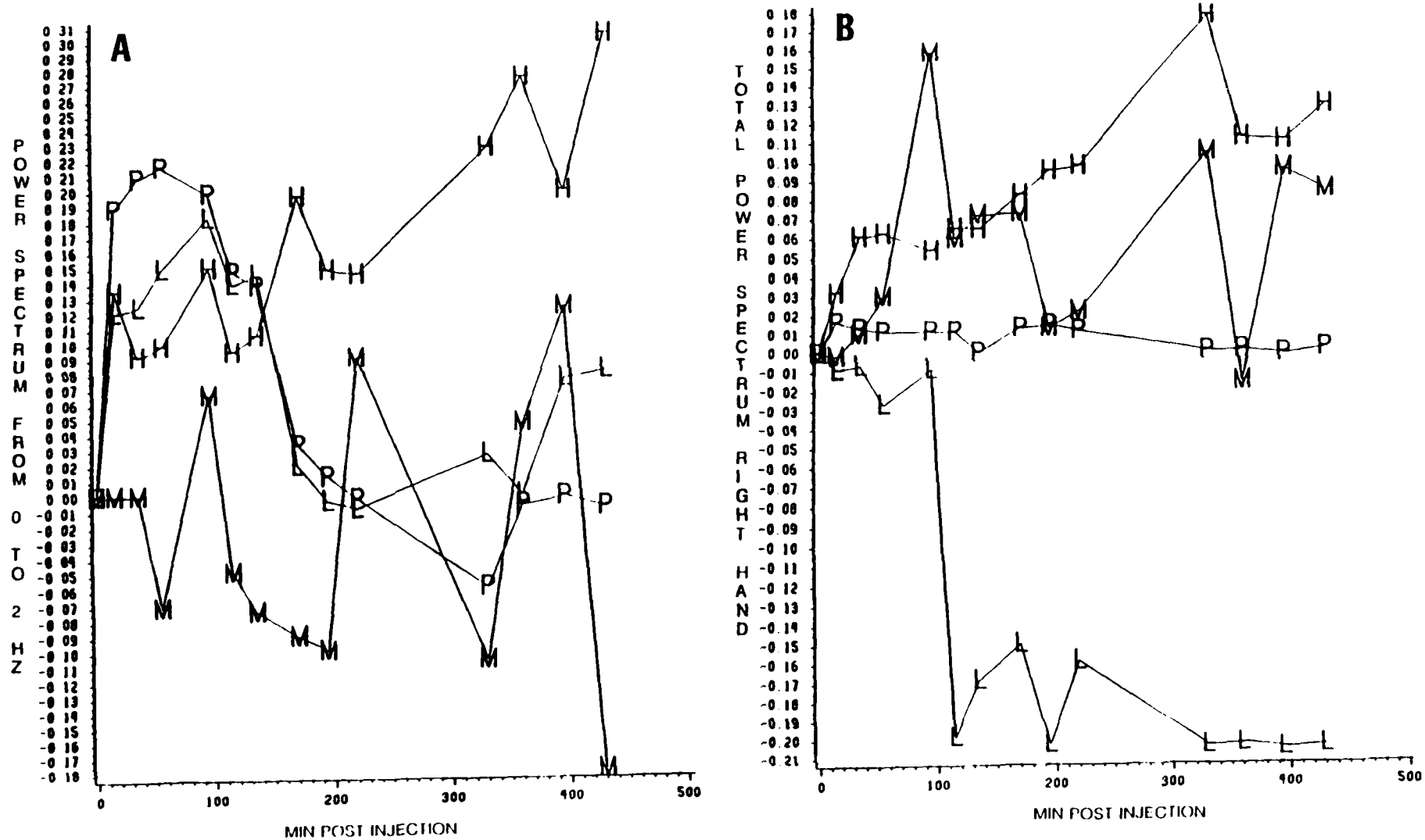

Figure 23. P = Placebo, L = 1.0 mg dose, M = 2.0 mg dose, and H = 4.0 mg dose.

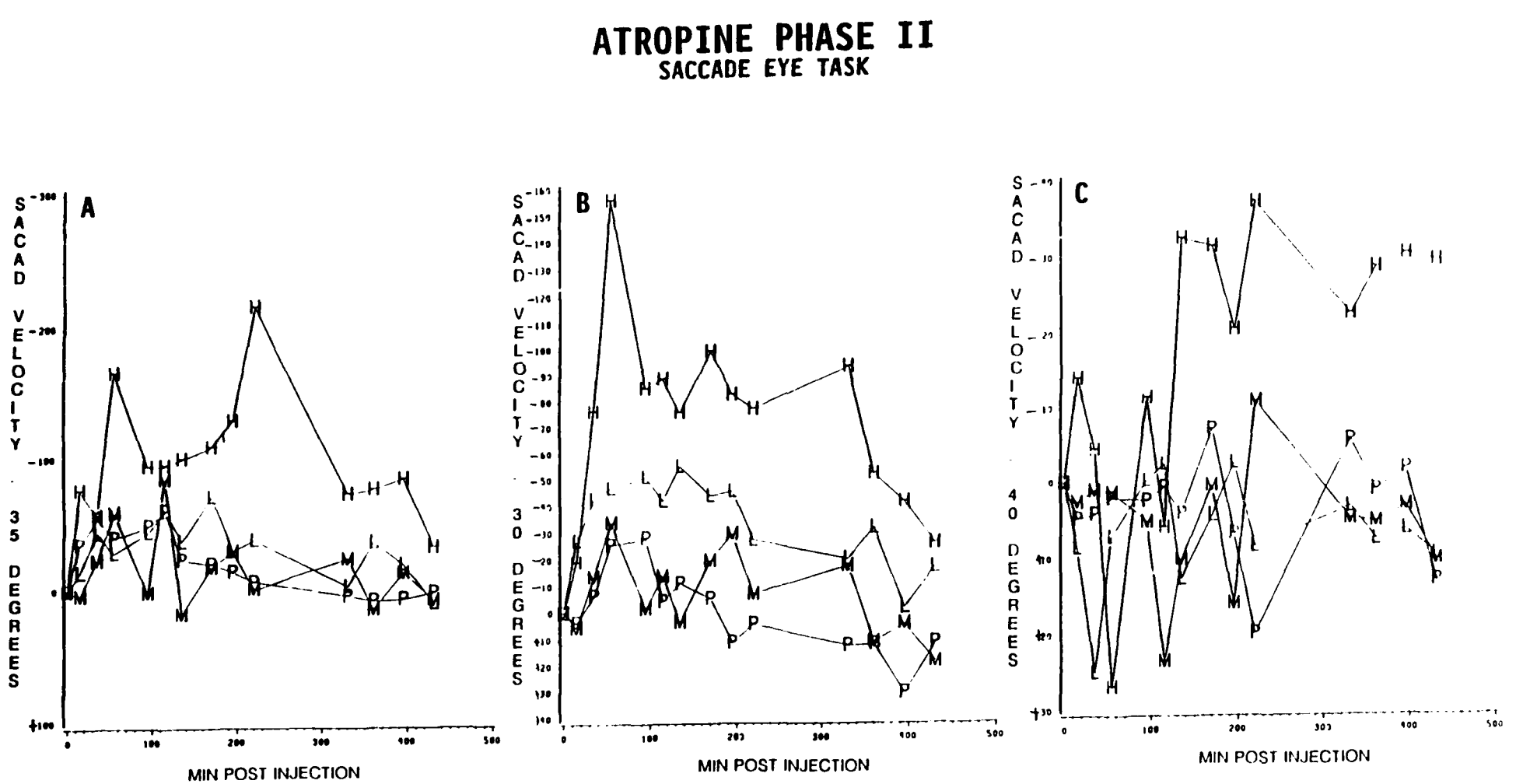

Figure 24. P = Placebo, L = 1.0 mg dose, M = 2.0 mg dose, and H = 4.0 mg dose.

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